UNIVERSIDAD COMPLUTENSE DE MADRID
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Impacto en las infecciones fúngicas invasivas de un hospital general de un programa de intervención diagnóstica, terapéutica y preventiva

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PRESENTADA POR

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El Profesor D. Emilio Bouza Santiago, Personal Emérito Asistencial del Servicio Madrileño de Salud y la Profesora Dña. Patricia Muñoz García-Paredes, Jefe del Servicio de Microbiología Clínica y de la Unidad de Enfermedades Infecciosas del Hospital General Universitario “Gregorio Marañón” y Catedrático de Microbiología de la Universidad Complutense de Madrid

CERTIFICAN:

Que el trabajo titulado “Impacto en las Infecciones Fúngicas Invasivas en un Hospital General de un Programa de Intervención Diagnóstica, Terapéutica y Preventiva” ha sido llevado a cabo bajo su dirección por Don. Antonio Vena y reúne las condiciones exigibles para ser presentado como tesis para aspirar a la obtención del título de Doctor por la Universidad Complutense de Madrid.

Para que conste y surta los efectos oportunos, firman este certificado en Madrid a 21 de septiembre de 2018.

Prof. E. Bouza Santiago
Director de la tesis

Prof. P. Muñoz García-Paredes
Co-directora de la tesis

INDICE

ABREVIATURAS

ADN Acido desoxirribonucleico

BDG 1-3 β-D-glucano

BC Blood cultures

CAGTA *Candida albicans* germ tube antibodies

C-CAN Complicated candidemia

CI Candidiasis invasora

CVC Catéter venoso central

E Especificidad

FDA Food and Drug Administration

GM Galactomanano

ICC Índice de Colonización Corregido

IFI Infección Fúngica Invasora

PCR Polimerasa Reacción en Cadena

S Sensibilidad

T2MR T2Candida Magnetic Resonance

UC-CAN Uncomplicated Candidemia

UCI Unidad de Cuidados Intensivos

UFC Unidad Formadoras de Colonia

VPN Valor Predictivo Negativo

VPP Valor Predictivo Positivo

I. INTRODUCCIÓN

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Las infecciones fúngicas constituyen un problema grave, que puede causar un gran número de entidades clínicas con manifestaciones muy variadas, que dependen del lugar de la infección y del tipo de pacientes [1]. En general, estas infecciones suelen clasificarse según un criterio de profundidad en micosis superficiales, cuando afectan sólo a tejidos como piel y uña y en micosis invasoras, cuando afectan a tejidos y órganos profundos.

Son las micosis invasoras las que, en los últimos años, han adquirido un protagonismo especial en los hospitales modernos, debido fundamentalmente a su elevada tasa de morbilidad y mortalidad y a su elevado consumo de recursos tanto para su prevención como para su manejo [2-4]. Además, estas infecciones constituyen un reto concreto y continuo para los clínicos ya que existen muchas incertidumbres que afectan aspectos diagnósticos, terapéuticos y preventivos [5, 6]

De hecho, hay muchas dudas sobre las indicaciones del tratamiento empírico y a la eficacia de los métodos diagnósticos precoces, incluyendo la dificultad para diferenciar entre colonización e infección invasora, cuando se dispone de aislados. Finalmente, en los últimos años, la población de pacientes susceptibles de desarrollar una infección fúngica invasora (IFI) se ha ampliado de forma significativa y han aparecido nuevos grupos de riesgo que están adquiriendo una importancia notable [7-10]

Por todo lo anterior resulta de especial importancia identificar las carencias en el conocimiento de las IFI para optimizar el diagnóstico de estas

infecciones, su manejo clínico y prevención. A estos tres aspectos intentaré aportar nuevos datos con en este trabajo

I.1 Importancia del problema de las infecciones fúngicas invasoras

En la actualidad, las infecciones fúngicas invasoras más frecuentes son aquellas producidas por *Candida* spp, *Aspergillus* spp, *Cryptococcus* y otros hongos filamentosos distintos de *Aspergillus*, tales como *Mucorales* y *Fusarium* [3, 4].

I.1.1 Candidiasis invasora

A pesar de que la distribución de los agentes causales varía en función del tipo de pacientes, de la geografía y de la unidad de hospitalización [2, 6, 11, 12]. las levaduras del género *Candida* constituyen la causa más frecuente de IFI en los hospitales modernos. Por ejemplo, en un estudio poblacional español en el que participaron 29 hospitales con 766 episodios, la incidencia de candidemia en la población general fue de 8.14 episodios por 100.000 habitantes (0,78 por 1.000 ingresos) [13]. De forma general, el 40-50% de los casos ocurrieron en las UCIs, pero serán también afectados gran variedad de pacientes ingresados en otros servicios médicos y quirúrgicos [11].

Datos similares se reflejan en la literatura, donde la incidencia de candidiasis invasora ha aumentado de forma significativa en los últimos 20 años [14]. El incremento de la incidencia está estrictamente relacionado con la mejora técnica de la medicina moderna y, por ello, los factores de riesgo más frecuentes son la cirugía abdominal, el uso de antibióticos de amplio espectro, la presencia de un catéter venoso central para nutrición parenteral, el desarrollo

de insuficiencia renal en los pacientes críticos, la neutropenia, la presencia de material protésico y el tratamiento inmunosupresor [15].

Desgraciadamente a pesar de los avances en el diagnóstico y en el tratamiento de las infecciones fúngicas invasoras, la mortalidad global continua siendo muy elevada, y en la cohorte española antes mencionada alcanza el 40% [11, 13].

I.1.2 Aspergilosis invasora

Entre los hongos filamentosos, *Aspergillus* tiene un gran interés y está emergiendo como un problema clínico mayor de la micología clínica moderna. Existen más de 20 especies de *Aspergillus* que, en mayor y menor medida, producen infección invasora en los humanos [16-18]. Sin embargo, en el 90% de los casos las especies implicadas son *Aspergillus fumigatus, Aspergillus flavus, Aspergillus niger,* y *Aspergillus terreus.*

La etapa inicial de la patogenia de este hongo es la inhalación de las esporas de *Aspergillus* que suelen dispersarse fácilmente en el aire y sobrevivir en diferentes condiciones ambientales [19]. A pesar del hecho que la inhalación de las esporas en el género humano es muy frecuente y en la mayoría de los casos no produce ninguna infección, en algunos huéspedes se puede producir una enfermedad invasora, que ha sido clásicamente descrita en los pacientes inmunodeprimidos.

El conocimiento sobre la incidencia real de aspergilosis en la población general sigue siendo escaso, ya que la enfermedad no suele sospecharse de

forma rutinaria [20] y que su diagnóstico suele ser muy difícil. En un reciente estudio Francés la incidencia de aspergilosis invasora pasó de 1,1 episodios/100,000 habitantes en 2001 a 1,8 episodios en 2010, con un aumento anual del 4.4% [21]. Datos similares han sido descritos en nuestro centro donde la incidencia ha subido de 0.08 casos/mil ingresos en 2014 a 0.66 casos en 2015. El incremento de la incidencia está principalmente relacionado con el hecho que el perfil de pacientes considerados de riesgo de aspergillosis invasiva está en expansión, habiéndose descritos casos de aspergillosis invasora en pacientes con menor grado de inmunosupresión aparentes, tales como los pacientes con enfermedad hepática aguda [7-10], pacientes ingresados en unidad de cuidados intensivos [22-24], EPOC [25], pacientes con gripe [26] o aquellos que reciben tratamientos con fármacos biológicos o con esteroides [27].

Aunque en la última década la tasa de supervivencia de los pacientes con aspergilosis invasora ha ido mejorando, la mortalidad relacionada con la API sigue siendo muy alta (mayor del 50%) en la mayoría de los casos [28-30]. La diminución de la mortalidad se debe principalmente al mejor pronóstico de los pacientes onco-hematologicos, tal vez influidas por los regímenes de acondicionamiento menos mielo-ablativos, los medios del diagnóstico más precoces y el empleo de regímenes de profilaxis más eficaces y mejor tolerados. Sin embargo, la disminución de la mortalidad no ha afectado a todos los grupos de pacientes. Por ejemplo, en un reciente estudio retrospectivo multicéntrico en el que se incluyeron 152 pacientes con aspergilosis invasora, la mortalidad relacionada con esta enfermedad fue del 67.4%, constatándose

que el peor pronóstico lo tenían las aspergilosis en los pacientes con enfermedad hepática [10]

I.1.3 Otras micosis oportunistas

Durante la década pasada, las IFI causada por levaduras raras y hongos filamentos distintos de *Aspergillus* se han incrementado en frecuencia y gravedad, con tasas muy elevadas de morbilidad y mortalidad (hasta un 90%), especialmente en los pacientes más inmunodeprimidos [31].

El incremento de la incidencia de estos hongos ha sido temporalmente vinculado a la introducción generalizada de la profilaxis con equinocandinas y voriconazol, fármacos que carecen de actividad contra los mucorales y levaduras raras [31]. De hecho, más que por el número absoluto de casos, la gran importancia reside en la virulencia de su comportamiento, en el grado de inmunosupresión del huésped y las pocas opciones terapéuticas disponibles.

Entre las otras micosis destacan en orden de frecuencias las siguientes familias o géneros: zigomicetos (*Mucor* spp., *Rhizospus* spp) [32], otros hongos filamentosos (*Fusarium* spp, *Scedosporium* spp), especie de *Cryptococcus* y otras levaduras [31, 33, 34].

I.2 El problema diagnóstico

Los pacientes susceptibles de sufrir una IFI son muy diversos: pacientes ingresados en UCI, neutropénicos, trasplantados de médula ósea y de órgano sólido, portadores de dispositivos intravasculares o que reciben nutrición parenteral o antimicrobianos de amplio espectro [15]. Por ello no es fácil diseñar estrategias diagnósticas aplicables a todas las situaciones en que ocurren las IFI [8].

Además, las manifestaciones clínicas de las IFI son inespecíficas y pueden estar enmascaradas por la enfermedad de base y por la situación clínica del paciente. Por otra parte, se estima que menos de la mitad de los pacientes que fallecen por una IFI han sido diagnosticados antes de la muerte, ya que la enfermedad no se suele sospechar clínicamente o que, con frecuencia, los cultivos nunca llegan a ser positivos [20].

Dado que el momento del inicio del tratamiento es determinante en el pronóstico [35, 36], muchas veces para iniciar tratamiento empírico el clínico ha de basarse esencialmente en su experiencia y en algunos “escores” clínicos, microbiológicos o mixtos basados en la presencia de diversos factores de riesgo y cuyo objetivo es permitir al clínico el reconocimiento de los pacientes con alto riesgo de desarrollar candidiasis invasiva (*Candida* score, Ostrosky score, ICC - Índice de Colonización Corregido) [37-39]. Estos escores son altamente específicos, pero tienen un valor predictivo positivo en torno al 5-10% [15]. Por ello, al usarlos indiscriminadamente, se termina administrando

tratamiento antifúngico innecesario a muchos pacientes que en realidad nunca tuvieron una candidiasis invasiva [15, 37]

Todo lo anterior ha conducido, como ya hemos mencionado, a un excesivo e indiscriminado uso de los antifúngicos, que no solo impacta en la evolución clínica de los pacientes, con aumento de las toxicidades relacionadas, sino también sobre el gasto ingente en su consumo y sobre el ecosistema hospitalario con aumento importante de la resistencia a los azoles y a las equinocandinas, que ya ha sido descrita en nuestro país [40, 41]. Si las cepas multi-resistentes terminan difundiéndose, la mortalidad de las micosis invasivas, podrá incrementarse de forma significativa. Por lo tanto, creemos que es necesario establecer nuevas estrategias diagnosticas basadas en medidas independientes de los cultivos que permitan acortar los tratamientos empíricos e identificar precozmente a los pacientes con CI.

I.3 Nuevas técnicas diagnósticas en el campo de las micosis invasoras

Durante los últimos años, con el intento de mejorar la aproximación diagnostica a las IFI se han desarrollado varios métodos para el diagnóstico de la enfermedad fúngica invasora que son independientes de cultivo y que paulatinamente se han ido popularizando, aunque no todas estas técnicas están definitivamente incorporadas en la práctica clínica diaria [5].

De forma general, se han introducido básicamente tres tipos de pruebas: las pruebas antigénicas, la detección de anticuerpos circulantes y las pruebas moleculares. Las pruebas antigénicas consisten en la detección de un componente de la pared celular del hongo en la sangre o en otros fluidos, como es la detección del galactomannano, 1,3-β-D-glucano (BDG); la detección de anticuerpos incluye los CAGTA (Candida albicans germ tube antibody) y los mananos-antimananos que se liberan durante la infección. Finalmente, las pruebas moleculares detectan el ADN del hongo en la sangre o en otras muestras clínicas [5].

I.3.1 Novedades en el diagnóstico de la candidiasis

El 1-3 β-D-glucano (BDG) es un biomarcador panfúngico, excepto para mucorales y *Cryptococcus* spp, que puede ser determinado bien en el suero bien en otras muestras biológicas. La detección en suero del BDG ha demostrado que puede ayudar a anticipar en 24 a 72 horas el diagnóstico de la CI en pacientes con candidemia [42]. Usando un punto de corte de 80 pg/ml la

prueba ha mostrado la siguiente eficacia diagnóstica: S= 92,9%, E=93,7%, VPP 72,2% y VPN 98,7%, mostrándose superior al *Candida* score y al índice de colonización. Pese a sus ventajas, deben considerarse también sus limitaciones incluyendo 1) el gran número de falsos positivos que presenta (hemodiálisis, colonización por *Candida*, bacteremias concomitantes, infusión de albumina o antibióticos [43] etc) 2) la escasez de datos sobre su cinética [44], haciendo imposible su uso como herramienta pronostica de evolución clínica.

CAGTA se ha desarrollado como técnica de inmunofluorescencia indirecta, que detecta IgG frente al antígeno hpw1 que se expresa en la pared celular de *Candida* en forma de hifa [45], permitiendo, por lo tanto, de discriminar entre colonización e infección. Esta técnica ha mostrado una medio-alta sensibilidad (42-96%) y especificidad (54-100%)[45-48], con buenos resultados en el diagnóstico de candidiasis invasiva, incluyendo pacientes con hemocultivos negativos.

Sin embargo, lo realmente útil de estas dos técnicas son sus elevados VPN, superiores al 90%, lo que permitiría descartar la presencia de infección fúngica con bastante certeza. En un estudio desarrollado en nuestro hospital[49] se evaluò la combinación de biomarcadores en la exclusión de candidemia, con la conclusión de que con la combinación de CAGTA con BDG se podía excluir candidemia con un VPN > 99.5% para prevalencias del 5-10%. Además, en un estudio prospectivo sobre pacientes que recibieron tratamiento antifúngico empírico por sospecha de candidiasis invasiva hemos demostrado que la negatividad de dos biomarcadores (CAGTA/ BDG) en los días 0, +3 y + 5

desde el inicio del tratamiento antifúngico empírico permite excluir el diagnostico de CI con un VPN del 100% en los pacientes ingresado en la UVI [50].

La detección combinada del antígeno manano y de los anticuerpos anti-mánanos es otra técnica serológica que ha sido empleada para el diagnóstico precoz de candidiasis invasora. Esta técnica sufre de falta de reproducibilidad en la detección del manano, así como la presencia de muchos falsos positivos y negativos y un gran número de resultados indeterminados [51].

Por otra parte, la detección del ADN de *Candida* a través de PCR alcanza una sensibilidad y especificidad de hasta el 100% en los casos de candidemia probada y del 95% y 92% en los pacientes con sospecha de candidiasis invasiva [52]. Además, estas técnicas permiten una identificación rápida de la especie de *Candida* [53]. A pesar de estas ventajas, hasta la fecha ninguna PCR ha sido validada para el diagnóstico de la candidiasis invasora y no hay evidencias concluyentes sobre la superioridad de ninguna de la tecnicas comerciales actualmente disponibles [5].

Finalmente, el T2 *Candida* es una nueva técnica diagnóstica aprobada por la FDA para el diagnóstico precoz de candidemia [54-56]. Es una técnica basada en la amplificación del ADN de *Candida* por medio de una polimerasa termoestable, seguida de la detección de los amplicones por medio de partículas nanomagneticas y resonancia magnética. Esta técnica es capaz de detectar directamente en la sangre la presencia de *Candida* con un límite de detección de 1-3 ufc. En un estudio piloto en el que se incluían 1500 pacientes

controles con hemocultivos negativos para *Candida*, 6 pacientes con hemocultivos positivos por *Candida* y 250 hemocultivos en los que se había inoculado una concentración conocida de *C. albicans*, *C. glabrata*, *C. parapsilosis* and *C. tropicalis* o *C.krusei*, la sensibilidad y especificidad de la técnica fue del 91% y 98% [55]. El tiempo necesario hasta obtener un resultado positivo o negativo del test fue sólo de 4.4 ± 1.0 horas [55]. Desafortunadamente, el conocimiento sobre la utilidad diagnostica de esta técnica en pacientes con sospecha clínica de candidiasis invasoras o su utilidad como herramienta pronostica es escaso.

I.3.2 Novedades en el diagnóstico de la aspergilosis invasora

Entre las nuevas técnicas utilizadas para el diagnóstico de la aspergilosis invasora, destaca el galactomananno (GM), un hetero-polisacarido termoestable presente en la pared celular de *Aspergillus*. La prueba comercial, que se denomina Platelia Aspergillus, es útil para la detección del GM en líquidos biológicos, tales como el suero y el lavado bronco-alveolar. La sensibilidad clínica del GM es muy variable con un rango de entre 22 y 100% [57], con una variación que depende de la enfermedad de base de los pacientes, siendo más alta (90%) en los pacientes hematológicos neutropénicos que no reciben profilaxis [57, 58]. Aunque el test ha sido valorado como test de screening para un diagnóstico precoz de aspergilosis invasora, publicaciones recientes demuestran que la prueba, utilizada como herramienta de screening es coste-

efectiva sólo cuando los pacientes no reciben profilaxis antifúngica y la prevalencia de la aspergilosis invasora supera el 7% [57, 58].

Desafortunadamente, hay muchas carencias en la literatura sobre estudios que hayan valorado el papel del GM como test de screening en otros contextos clínicos, como en pacientes no hematológicos o pacientes hematológicos que reciben profilaxis antifúngica. Finalmente, en los últimos años se ha valuado la detección del GM en el lavado bronco-alveolar (LBA), logrando muy buenos resultados con una sensibilidad de hasta el 100% y con una especificidad entre el 75 y 92% [59].

Teniendo en cuenta su alto valor predictivo negativo, el test del BDG podría tener un papel en algunos algoritmos diagnósticos con el fin de excluir más que confirmar, el diagnóstico de aspergilosis invasora. De hecho, un resultado positivo del BDG en sí mismo no es diagnóstico de la enfermedad y requiere la realización de otras pruebas complementarias [8].

A pesar del hecho que algunos meta-análisis apoyan el uso de la PCR en sangre para excluir el diagnóstico de la AI en pacientes inmunocomprometidos [60], las pruebas moleculares de amplificación de los ácidos nucleicos no están estandarizadas para *Aspergillus* y los datos son insuficientes para recomendar esta técnica como método diagnóstico habitual.

En resumen, ninguna de las técnicas diagnósticas mencionadas tiene una sensibilidad suficiente como para permitir individualmente el diagnóstico de aspergilosis invasiva. Por lo tanto es la combinación de varios biomarcadores lo que parece aumentar el diagnóstico de esta enfermedad, precediendo el

desarrollo de enfermedad manifiesta y permitiendo un uso racional de los agentes antifúngicos [8, 43].

I.4 El problema terapéutico

Como ya hemos mencionado, el tratamiento de las micosis suele comenzarse empíricamente, basándose en los factores de riesgo del paciente y en la epidemiologia local [61]. En este sentido el empirismo inicial está justificado tratándose de pacientes graves, con un riesgo real de desarrollar una micosis, en los que se ha comprobado que el inicio precoz del tratamiento antifúngico tiene un gran impacto sobre la supervivencia [62-64].

En estudios previos hemos comprobado que el tratamiento empírico supone el 90% de los antifúngicos que se suelen pautar en una UCI y el 60% de todos los antifúngicos que se han pautado en un hospital [61]. En estos mismos estudios hemos podido observar que incluso médicos muy formados, como son los médicos que se dedican a los pacientes trasplantados, o lo de la UCI, tienen un alto grado de incumplimiento de las guías y de uso innecesario de antifúngicos [65]. Un trabajo previo hecho en nuestro hospital muestra que el problema esencial no es la indicación de comenzar un tratamiento antifúngico que es correcta en el 96% de las ocasiones, sino la duración del mismo [61]. De hecho resulta ser complicado suspender un fármaco en un paciente crítico cuando no se dispone de un diagnóstico alternativo. Para poder solucionar esta dificultad, hemos llevado a cabo un estudio prospectico, previamente citado, en el que la combinación de los biomarcadores analizados en los días 0,+3,+5 nos permitió de identificar una estrategia capaz de suspender de forma segura tratamientos antifúngicos innecesarios [50]. Desafortunadamente, esta aproximación diagnóstica necesita como mínimo de 5 días de tratamiento

antifúngico y no incluye otras técnicas recientemente aprobadas para el diagnóstico de la candidemia como la resonancia magnética.

Otros aspectos todavía pocos explorados y que afectan la elección del tipo de antifúngico a administrar son:

1. **Biodisponibilidad en el órgano o tejido afectado.** Por ejemplo el SNC y el humor vítreo son lugares de difícil acceso debido al gran tamaño de algunos antifúngicos o a la elevada unión aproteínas plasmáticas [15]; en este sentido prácticamente no hay datos sobre la evolución clínica de pacientes con candidiasis ocular que reciben tratamiento con candinas, antifúngicos de primera línea que pero alcanzan bajas concentraciones en estos lugares.
2. **Tolerancia y toxicidad** Pese a que los nuevos antifúngicos son fármacos muy bien tolerados, hasta la fecha prácticamente hay muy pocos datos sobre la tolerancia y la toxicidad, especialmente en nuevas poblaciones de riesgo, cual son los pacientes con hepatopatía previa en fase avanzada.
3. **Variabilidad en los niveles de antifúngicos en sangre**. Al momento actual no hay estudios que hayan valorado la variabilidad interindividual de los niveles de antifúngicos en sangre. Asimismo, no hay datos sobre la necesidad de medir niveles en poblaciones concretas, tales como niños o pacientes en hemodiálisis, ni sobre el impacto clínico que pueda tener medir de forma sistemática los niveles de antifúngicos en pacientes hospitalizados.

I.5 El problema de la prevención

Como ya hemos mencionado, la carga global de enfermedad causada por las IFI y su impacto sanitario es muy importante, tanto en mortalidad cuanto en gastos hospitalarios. Las limitaciones aún existentes en los métodos diagnósticos actuales para conseguir un diagnóstico precoz de IFI y la emergencia de patógenos fúngicos con resistencia a algunos de los antifúngicos disponibles, hacen que las medidas de prevención de las IFI hayan adquirido una relevancia muy importante.

Es evidente que estas estrategias de control de la infección son diferentes según el objetivo sea prevenir las IFI causadas por *Candida* o por hongos filamentosos.

I.5.1 Prevención de la infecciones por *Candida*.

En general, las estrategias dirigidas a la prevención de la candidiasis se fundamentan en el posible origen fisiopatogénico de su modo de aparición: origen “exógeno“(por ejemplo, CVC) o el origen “endógeno” (colonización del tracto digestivo).

Las estrategias en la prevención de las fungemias originadas en los catéteres se basan en procesos esenciales como son principalmente la higiene rigurosa de manos, la antisepsia con clorhexidina, las precauciones óptimas de barrera, el uso preferente de implantación del CVC por vía subclavia, y la retirada de aquellos CVC no necesarios [66].

Por otra parte, en las candidemias “endógenas” confluyen tres elementos esenciales en su promoción, como son: 1) la presión selectiva ejercida por el empleo prolongado de antibióticos de amplio espectro; 2) la colonización progresiva de distintos aparatos por especies de *Candida*, especialmente la de tracto gastrointestinal; 3) la afectación del estado inmunitario del huésped, tanto local (cirugía) como general (inmunosupresión) [67].

En estas situaciones las medidas que tienen un valor más importante son aquellas que incluyen la optimización de los procedimientos quirúrgicos, el manejo adecuado de la inmunosupresión y sobre todo el correcto empleo de antimicrobianos [67].

Además, en la última década se ha ampliamente discutido la posibilidad de instaurar una estrategia de profilaxis antifúngica en pacientes “ad alto riesgo” [68, 69], aunque si evidencias recientes no apoyan el uso de profilaxis primaria en ninguna condición (excepción hecha por los pacientes trasplantados), ya que no se ha identificado un subgrupo de pacientes en los que esta estrategia es rentable y coste-efectiva [15].

I.5.2 Prevención de la aspergilosis invasora.

Como ya mencionado, en los últimos años la supervivencia global de los pacientes con aspergilosis invasora ha ido mejorando en parte por el gran esfuerzo que se ha puesto en la prevención de la las infecciones fúngicas, mediante la utilización de estrategias de profilaxis.

Actualmente, el uso de la profilaxis antifúngica se ha demostrado eficaz en los pacientes con neoplasia hematológica de elevado riesgo y pacientes trasplantados de progenitores hematopoieticos [69]. En otros grupos de pacientes, tales como los trasplantados de órgano solido la profilaxis no está bien establecida y depende del tipo de órgano, nivel de inmunosupresión, presencia de alteraciones de la defensa locales secundarias al trasplante [70].

Sin embargo, es necesario considerar que la eficacia en la reducción de las infecciones por hongos filamentosos se debe también a otras medidas, quizá más importantes, cuales son el control ambiental mirado a evitar la exposición aérea a esporas de hongos filamentosos y el correcto manejo de los esteroides y de la infección hospitalaria [71].

I.6 Impacto económico

Las infecciones fúngicas invasoras no solo tienen un gran interés epidemiológico, tienen también un gran impacto económico. Por ejemplo el coste de un episodio de candidemia se ha estimado en 44.000 dólares en adultos, mientras que el conste de un episodio di aspergilosis invasora se ha estimado entre 48.000 y 84.000 dólares [72]. Existe además una gran diferencia entre el número de episodios anuales de micosis probadas, que en un hospital terciario como el nuestro, son entre 60 y 100 al año, y el gasto farmacéutico en antifúngicos que ha crecido exponencialmente en el curso de los últimos 10 años. Esto se debe a la aparición de nuevos antifúngicos más eficaces, con mejor espectro de acción, mejor tolerados pero mucho más costosos (un ciclo de tratamiento con una equinocandina supone aproximadamente 6.400 euros frente a los 240 euros que costaría el mismo curso con fluconazol) y a los tratamientos empíricos en pacientes sin diagnóstico establecido.

II.ALGUNAS CARENCIAS EN LA LITERATURA

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Como ya hemos mencionado, en los últimos años, se han introducido nuevas herramientas para el diagnóstico de la IFI, como la resonancia magnética T2Candida y los biormacadores. La rentabilidad de estas técnicas, sin embargo, ha sido únicamente analizada en estudios que incluyeron pocos pacientes con candidiasis invasora. Además, hasta la fecha no hay estudios que evalúen el valor de estas pruebas para monitorizar las complicaciones y la respuesta clínica en los pacientes con candidemia.

Monitorizar los pacientes con candidemia es muy importante ya que dicha enfermedad está asociada con una alta tasa de complicaciones metástasicas y con una elevada mortalidad. El diagnóstico precoz, la administración temprana de un tratamiento antifúngico adecuado y el control apropiado del origen de infección han demostrado ser factores clave que mejoran el pronóstico de los pacientes con candidemia. Sin embargo, el impacto de estos factores claves no ha sido evaluado como parte de un modelo de paquete de medidas, y las intervenciones específicas no siempre se cumplen a tiempo. Con respecto a este tema, hay pocos trabajos en los que se valora la implementación sistemática de recomendaciones estructuradas, dirigidas a mejorar la adherencia a las guías de candidemia. Además, los trabajos existentes incluyen pocos pacientes y fueron realizados en hospitales pequeños, muchos de los cuales no habían previamente implementado un programa de optimización del uso de antifungicos como el nuestro.

Un tratamiento antifúngico adecuado requiere la selección y dosificación adecuada de la molécula. Desafortunadamente, hasta la fecha no se considera rutinariamente la necesidad de monitorización terapéutica de estos fármacos, principalmente debido a la creencia de que la concentración sérica antifúngica adecuada generalmente se logra prescribiendo dosis fijas de acuerdo con las guías internacionales [73]. Sin embargo, las evidencias que respaldan esta suposición son escasas y principalmente relacionadas con grupos de pacientes específicos o clases de antifúngicos.

Debido a la alta mortalidad asociada con la IFI, la profilaxis constituye un elemento fundamental para disminuir la misma. La detección sérica de galactomanano se usa ampliamente como una prueba de “screening” en pacientes de alto riesgo, porque permite el diagnóstico precoz de aspergilosis invasora, mejorando así la supervivencia [74, 75]. Estudios recientes han demostrado que durante la profilaxis con posaconazol, la determinación de GM en suero sigue siendo útil solo para el diagnóstico de pacientes sintomáticos con sospecha clínica de AI, ya que los resultados en pacientes asintomáticos fueron tanto negativos como falsos positivos [76]. Sin embargo, en los últimos años, la micafungina ha sido el fármaco profiláctico que se ha utilizado con más frecuencia, especialmente en pacientes con el mayor riesgo de desarrollar IFI, como son los pacientes con mucositis. Desafortunadamente, ningún estudio ha investigado el valor diagnóstico del GM sérico en pacientes que reciben profilaxis con micafungina. además, hay muy pocos estudios que hayan descrito la tasa de IFI de brecha en esta población.

III. OBJETIVOS

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Primer objetivo

1. Describir la incidencia, factores clínicos predisponentes y la evolución de los pacientes con candidemia complicada en tres grandes hospitales de la Comunidad de Madrid.
2. Evaluar si en comparación con los hemocultivos tradicionales y el (1→3)-beta-D-glucano, la determinación seriada de T2MR es capaz de discriminar entre candidemia complicada y no complicada.

Segundo objetivo

1. Evaluar la utilidad clínica de los biomarcadores de candidiasis invasiva y del T2MR usados prospectivamente en pacientes con sospecha de candidiasis invasiva que reciben tratamiento empírico para la retirada precoz de los tratamientos antifúngicos innecesarios

Tercer objetivo

1. Describir en un hospital terciario con gran experiencia en política de antifúngicos, el impacto clínico que puede tener la aplicación sistemática de un paquete compuesto por medidas diagnósticas y terapéuticas sobre la morbi-mortalidad de los pacientes con candidemia.

Cuarto objetivo

1. Definir si existe asociación entre la dosis de fármacos antifungicos y la concentración sérica adecuada en un grupo no seleccionado de pacientes hospitalizados.
2. Evaluar el impacto clínico de una dosis inadecuada o concentraciones séricas inadecuadas en el desenlace clínico de los pacientes con infección fúngica invasiva.

Quinto objetivo

1. Evaluar la eficacia, tolerabilidad y seguridad del tratamiento antifúngico con micafungina en pacientes con insuficiencia hepática severa.
2. Definir si existe asociación entre exposición a micafungina y desarrollo de neoplasia hepática a largo plazo.

Sexto objetivo

1. Investigar la rentabilidad diagnostica del galactomannano sérico en pacientes hematológicos de alto riesgo que reciben profilaxis con micafungina.
2. Describir la tasa de aspergilosis invasora de brecha en esta población.

A partir de esto momento, dada la diversidad de los objetivos de esta tesis y para hacer más claros los mensajes de los distintos trabajos, he decido agrupar los datos globales de cada objetivo por separado, manteniendo el modelo clásico de tesis doctoral para cada uno de ellos.

IV. PRIMER OBJETIVO:

Contribución del T2MR al diagnóstico temprano de la candidemia complicada.

Evaluation of T2MR for the very early diagnosis of complicated candidemia

INTRODUCTION

Candida bloodstream infections (BSI) are frequently associated with metastatic complications [77-80] and with high mortality rates, ranging from 25% to 40% [81-83]. Early differentiation between complicated and uncomplicated disease represents a priority in the management of candidemia because (complicated candidemia [C-CAN]) requires longer antifungal therapy and more aggressive control of infection source [15, 84].

Differentiation between complicated and uncomplicated candidemia (UC-CAN) is usually based on the persistence of positive blood cultures (BCs) during follow-up [15, 84]. However, the sensitivity of follow-up *Candida* BCs is only about 50%[85], and it further decreases when adequate antifungal therapy is started, thus making BCs a far from ideal test for monitoring complications and outcome during candidemia [86-88]. T2 *Candida* magnetic resonance (T2MR) is a new diagnostic method for diagnosis of candidemia, approved by the US FDA [55] and by the EMEA. In a recent clinical trial involving 1,801 hospitalized patients, T2MR was effective in detecting the five most common species of *Candida* in BCs, with an overall specificity of 99.4% and sensitivity of 91.1% [55]. However, T2MR has not been yet evaluated as a follow-up instrument to distinguish episodes of C-CAND and UN-CAND.

The objective of this prospective multicenter study was to assess the usefulness of follow-up T2MR in comparison to BCs and β-D-glucan assay (BDG) to early differentiate patients with complicated or uncomplicated *Candida* BSI.

MATERIAL AND METHODS

Study population and setting

This first study was performed between January and June 2017 in 3 centers in Madrid. Patients (≥ 18 years) with BSI caused by *Candida* species were included in the study if they gave their informed consent and were monitored for at least 1 month or until death.

The local ethics review committees approved the study protocol, and written informed consent was obtained from each patient (Number of ethical committee approval MICRO.HGUGM.2016-023)

Study Design and tests performed

As soon as the first positive BC yielding *Candida* species was detected (index positive blood culture - Day 0), the site investigator obtained the patient's consent and managed each case according to routine clinical care, until death or hospital discharge. A transesophageal echocardiogram and a dilated ophthalmoscopy were systematically recommended at baseline for all patients included in the study. The search for other septic metastases was performed when clinically indicated.

Blood samples were obtained at day 0, day +2, day +7 and day +14 to perform BC, T2MR and BDG, simultaneously, until all tests were negative. In all hospitals, only the results of the BC were available to clinicians. Both T2MR and BDG results were provided at the end of the study and had no influence on the clinical management of the patients.

Microbiological samples

BCs were obtained through venepuncture using standard procedures and processed in the clinical laboratories with the BD Bactec FX (Becton, Dickinson, Sparks, MD) at Hospital Gregorio Marañón and Hospital Ramon y Cajal and with the BacT/Alert 3D (Biomerieux) at Hospital 12 de Octubre.

T2MR and the BDG assay were processed twice weekly at the clinical laboratory of the coordinating center (Hospital General Gregorio Marañón). The T2MR (T2 Biosystems Inc.) was performed in EDTA whole-blood samples obtained from a peripheral vein and processed automatically following all the steps previously described by Mylonakis et al.[55] T2MR results were grouped based on antifungal resistance patterns of different *Candida* species,[15] and reported as follows: *C. albicans*/*C. tropicalis*, *C. krusei*/*C. glabrata*, and *C. parapsilosis*. If the internal control was invalid and there were no positive T2MR signals, an "invalid" result was displayed, indicating that the specimen may contain inhibitors that interfered with *Candida* detection [55].

Serological detection of BDG was performed using the Fungitell assay kit (Associates of Cape Cod) according to the manufacturer's instructions, using a cutoff of 80 pg/ml. Results were read and analyzed with a BioTek ELX808TM Microplate Reader and GEN5 Software (BioTek U.S., VT, USA) and considered positive when the values were ≥ 80 pg/mL [50].

Clinical data

The following data were prospectively collected using a standardized case report form: age, sex, underlying disease, Charlson's comorbidity index; risk factors for candidemia (i.e. presence of central venous catheter, parenteral nutrition, corticosteroid therapy, recent surgery, previous *Candida* colonization); clinical presentation; source of fungemia; *Candida* species; type of antifungal therapy and dosage; presence of septic metastasis; source control of infection, and clinical evolution of the patient.

***Definition of complicated candidemia* (C-CAN)**

C-CAN was defined as all the episodes involving metastatic spread to other organs (i.e. endocarditis, other endovascular infections, ocular candidiasis, etc.), and/or with attributable mortality during the follow-up period.

Other definitions

An episode of candidemia was defined as a patient that had at least 1 peripheral blood culture positive for *Candida* species As for the underlying diseases, we classified patient situations as rapidly fatal, ultimately fatal, or non-fatal according to the classic criteria of McCabe and Jackson.[89] Sepsis, septic shock, and Pitt's bacteraemia score were defined according to standard international criteria [90, 91].

As for the source of infection, an episode of candidemia was considered as catheter-related if 1) the catheter tip culture was positive with the same *Candida* species, 2) there was evidence of catheter exit site exudate with the

same *Candida* species or 3) the differential time to positivity of BCs obtained from the catheter and peripheral veins was greater than or equal to 2 hours.[92]

The urinary tract was considered to be the portal of entry in patients with urologic predisposing conditions (i.e. manipulation or obstruction of the urinary tract) and evidence of urinary tract infection caused by the same species of *Candida*.

The abdomen was considered to be the origin of the candidemia when a patient had evidence of abdominal infection and 1) a positive culture from the intra-abdominal space was obtained during surgery or by needle aspiration and/or 2) no other apparent sources of candidemia where detected. When a source of candidemia could not be identified, candidemia was defined as "primary".

Patients were considered to have *Candida* septic metastasis when an infection due to the same *Candida* species occurred in a site that was distant from the source of the candidemia. In cases in which no culture was available, the distant infection had to be temporally related with the fungemia and with no alternative cause explaining the clinical condition.

Infective endocarditis was diagnosed according to Duke's criteria;[93] ocular candidiasis was classified based on previous criteria;[77, 79] septic thrombophlebitis required the presence of venous thrombosis, confirmed by imaging techniques, in the setting of persistent candidemia.[92] Mortality was considered attributable to candidemia in patients who died with persistent positive BCs for *Candida* species or with persistent signs or symptoms of

candidemia. Doubtful cases were independently reviewed by three investigators blind to T2MR and BDG results (AV, MM, PM); unanimous agreement was required.

Statistical analysis

Sensitivity, specificity and predictive values were calculated for predicting complicated candidemia for: 1) BCs; 2) T2MR; and 3) BDG at different times (from day 0 to day 5 [early period] and beyond day 5 [late period]). The timeframes were chosen because they represent the period in which follow-up BCs are usually performed in real clinical practice. Non-normally distributed continuous variables were compared using the t-test, and normally distributed variables were compared using the t-test or analysis of variance. Stepwise logistic regression models were applied in the multivariate analysis to control for potential confounders and for risk factors of C-CAN. Variables with a p-value <0.1 in the univariate analysis were included in the multivariate models. Differences were considered to be significant for $p < 0.05$. The analysis was carried out with SPSS 18.

RESULTS

During the study period, 44 patients were diagnosed with an episode of candidemia in the 3 Institutions. Of those, 6 patients died within the first 2 days, 5 were excluded because they refused to participate in the study, and 3 had invalid T2*Candida* MR results (one patient from each participating hospital).

Thus, 30 patients were included in the final analysis and are the object of the present study. The main pathogens were *Candida albicans*, *Candida parapsilosis*, *Candida glabrata*, and *Candida tropicalis*, which were isolated in 43.3%, 30.0%, 16.7%, and 6.7% of patients, respectively. Fundoscopies and echocardiograms were performed in 86.7% and 76.3% of patients, respectively.

Clinical characteristics of complicated and uncomplicated Candida BSI

Nine out of the 30 patients (30.0%) fulfilled the criteria for complicated candidemia whereas 21 (70.0%) were classified as having uncomplicated disease. Clinical characteristics of the 9 patients with complicated candidemia are shown in **Table 1**. The most frequent complications were ocular candidiasis in 5/9 (55.6%) and infective endocarditis in 2/9 patients (22.2%).

Table 1. Clinical characteristics of the 9 patients with complicated candidemia

Age /Sex	Charls on's CI	Main underlying disease	*Candida* sp.	Source of candidemia	Type of complication	Related Mortality	Early (d 1-5)			Late (> d 5)		
							BC	BDG	T2	BC	BDG	T2
73/F	2	Cardiovascular disease, esophagus cancer receiving chemotherapy	*C. albicans*	Central venous catheter	Ocular candidiasis, Septic thrombophlebitis	No	+	+	+	+	+	+
60/M	3	Chronic obstructive pulmonary disease, tongue cancer	*C. tropicalis*	Port-a-cath	Tricuspid endocarditis Lung metastasis	No	-	+	+	+	+	+
74/M	3	Neurological disease, diabetes, prostate cancer, major	*C. parapsilosis*	Abdomen	Ocular candidiasis	No	-	+	+	-	+	-

		abdominal surgery for complicated cholecystectomy										
59/F	7	Cardiovascular disease, chronic renal insufficiency, diabetes, gastrointestinal disease	*C. albicans*	Central venous catheter	Ocular candidiasis	No	-	+	-	-	+	-
52/M	5	Neurological disease, chronic obstructive pulmonary disease, liver transplant	*C. albicans*	Abdomen	Ocular candidiasis	No	-	+	+	-	+	-
84/M	6	Chronic renal insufficiency, esophageal stenosis. chronic obstructive pulmonary disease	*C. albicans*	Central venous catheter	No septic metastasis	Yes	+	+	+	NP	NP	NP
41/M	0	No underlying disease. Patients admitted to	*C. albicans*	Central venous	Ocular candidiasis	No	-	+	+	-	+	+

		ICU for severe community acquired pneumonia		catheter								
63/F	7	Chronic renal insufficiency, cardiovascular disease, diabetes mellitus	*C. albicans*	Central venous catheter	No septic metastasis	Yes	+	+	+	NP	NP	NP
77/M	7	Chronic renal insufficiency, cardiovascular disease, diabetes mellitus	*C. parapsilosis*	Unknown	Aortic valve endocarditis	No	+	+	+	-	+	+

CI comorbidity index; d: day; **F** female; **ICU** Intensive Care Unit; **M** Male; **NP** Not performed

The main epidemiological characteristics and clinical manifestations in uncomplicated and complicated candidemia are compared in **Table 2**. Age, sex, underlying disease, risk factors for candidemia, BSI source and etiology were similar between groups. However, patients with complicated disease were more likely to have sepsis at the time of *Candida* BSI (55.6% versus 9.5%, p=0.01). No further differences were found regarding Pitt score, adequate source control of infection, length of hospital stay, and in-hospital mortality.

Table 2. Comparison of clinical features between patients with uncomplicated or complicated candidemia

VARIABLES	Uncomplicated (n=, 21%)	Complicated (n=, 9%)	*p*
Age, years, (mean ± SD)	66.3 ± 14.3	64.8 ± 13.5	0.79
Sex, male	11 (52.4)	6 (66.7)	0.69
Underlying disease			
Malignancy	11 (52.4)	3 (33.3)	0.44
Diabetes	9 (42.9)	4 (44.4)	1
Cardiovascular	7 (33.3)	4 (44.4)	0.68
Gastrointestinal disease	6 (28.6)	3 (33.3)	1
Chronic liver disease	4 (19.0)	1 (11.1)	1
Chronic renal disease	3 (14.3)	3 (33.3)	0.32
Mc Cabe score			
Non-fatal	11 (52.4)	3 (33.3)	0.44
Ultimately fatal	10 (47.6)	6 (66.7)	0.44
Charlson comorbidity index	3.2 ± 1.9	4.4 ± 2.5	0.16
Risk factors for candidemia			

CVC in site	14 (66.7)	6 (66.7)	1
Total parenteral nutrition	9 (42.9)	6 (66.7)	0.42
Previous surgery, previous 90 days	9 (42.9)	4 (44.4)	1
Corticosteroid therapy, previous 30 days	4 (19.0)	3 (33.3)	0.64
Antibiotic therapy, previous 30 days	16 (76.2)	7 (77.8)	1
BSI source			
Catheter related	13 (61.9)	7 (77.8)	1
Abdominal	3 (14.3)	2 (22.2)	0.62
Urinary tract infection	3 (14.3)	0 (0)	0.53
Primary	3 (14.3)	0 (0)	0.53
Etiology			
C. albicans	7 (33.3)	6 (66.7)	0.12
C. parapsilosis	7 (33.3)	2 (22.2)	0.68
C. glabrata	5 (23.8)	0 (0)	0.28
C. krusei	1 (4.8)	0 (0)	1
C. tropicalis	1 (4.8)	1 (11.1)	0.51
Severity of *Candida* BSI			
Sepsis	2 (9.5)	5 (55.6)	**0.01**
Septic shock	3 (14.3)	1 (11.1)	1
No sepsis	16 (76.2)	3 (33.3)	**0.04**
Pitt Score, median [IQR]	2 (0-3.5)	1 (0-2.5)	0.76
ICU admission	3 (14.3)	2 (22.2)	0.62
Adequate source control of infection	15 (71.4)	7 (77.8)	0.85
Days of AF therapy before T2MR, median [IQR]	2 (1.0-4.5)	3 (1.5-3.0)	0.44
Days between index BC and first T2 *Candida* MR, mean ± SD	3.7 ± 1.4	3.6 ± 1.3	0.77
Days of hospitalization, median [IQR]	45.5 (38.0-59.3)	37.0 (15.0-48.0)	0.82
In hospital mortality	1 (4.8)	3 (33.3)	0.07

Total days of AF therapy, median [IQR]	18 (15.0-32.0)	39 (8.0-67.3)	0.22

IQR Inter-Quartile Range

Positivity rates for BCs, T2*Candida* MR and β-D-glucan

The median time of appropriate antifungal treatment before obtaining the first blood sample processed for follow-up BC, T2MR and BDG was 2 days (IQR), with no differences between UN-CAN and C-CAN (2 versus 3 days, p=0.44).

Overall, 116 follow-up T2MR samples, 111 BDG samples and 69 BCs sets were collected during the study period. Of these, 82 T2MR, 80 BDG and 58 BCs were collected during the first 5 days (early period) whereas the remaining 34, 31 and 11, respectively were obtained beyond day 5 (late period).

Positivity of T2MR, BC and BDG assay in the early period was respectively: 32/82 (39.0%), 14/58 (24.1%) and 57/80 (71.3%). Beyond day five (late period), the positivity rates of the three studied tests were: 7/34 (20.6%), 3/11 (27.3%) and 26/31 (83.9%). There were 8 invalid T2MR results (6.9%).

During the early period, follow-up evaluation with BCs, T2MR and BDG showed that all tests were already negative in 6/30 patients (20%), whereas they resulted all positive in 6/30 (20%) patients. Discordant results were obtained for the remaining 18/30 (60%) patients. In most cases, discordant results consisted either in a positive T2MR and BDG assay with negative BCs

(7/30, 23.3%) or in a positive BDG assay with negative T2MR and BCs (7/30, 23.3%).

The diagnostic value of the early BCs, T2MR and BDG for predicting C-CAN are shown in **Table 3.** Patients with a C-CAN were more likely to have a positive T2MR, both in the early [5/21 (23.8%) patients with positive T2MR versus 9/9 (100%) patients with negative T2MR, p=<0.001) and in the late period [1/19 (5.3%) patients with a positive T2MR versus 6/7 (85.7%) patients with a negative T2MR, p=0.001]. Neither BCs nor BDG could differentiate episodes of complicated and uncomplicated candidemia during the early period.

Table 3 also shows the sensitivity, specificity and predictive values of all three techniques for predicting complicated candidemia. In the early period, T2MR showed a higher sensitivity than BCs for predicting complicated candidemia, and both had similar specificities. In the late period, the T2MR continued to remain positive longer than BC, but less than the BDG assay. However in the late period BDG showed a very low specificity (33.3%).

Table 3. Yield of the three studies tests for the prediction of complicated and uncomplicated candidemia.

	Test	Uncomplicated (n=21, %)	Complicated (n=9; %)	*p*	S (95% CI)	Sp (95% CI)	PPV (95% CI)	NPV (95% CI)
EARLY SAMPLEs (<5 d)	**Positive BC**	5 (23.8)	4 (44.4)	0.39	44.4 (15.3-77.3)	76.1 (52.4-90.8)	44.4 (15.3-77.3)	76.1 (52.4-90.8)
	Positive T2 MR	5 (23.8)	9 (100)	**<0.001**	100 (62.8-100)	76.1 (52.4-90.8)	64.2 (35.6-86.0)	100 (75.9-100)
	Positive B-DG	13 (61.9)	9 (100)	0.07	100 (62.8-100)	38.9 (18.9-61.3)	40.9 (21.5-63.3)	100 (59.7-100)
LATE SAMPLEs (>7 d)	**Positive BC***	0/6	2/5 (40.0)	0.18	40.0 (7.2-82.9)	100 (51.6-100)	100 (19.7-100)	66.7 (30.9-90.9)
	Positive T2 MR[#]	1/19 (5.3)	6/7 (85.7)	**0.001**	85.7 (42.0- 99.2)	94.7 (71.9-99.7)	85.7 (42.0- 99.2)	94.7 (71.9-99.7)
	Positive BDG[&]	12/18 (66.7)	7/7 (100)	0.14	100 (56.0-100)	33.3 (14.3-58.9)	36.8 (17.2-61.3)	100 (51.6-100)

*Test performed in 11 patients; [#] Test performed in 26 patients [&] Test performed in 25 patients

BC: blood culture; BDG: beta D glucan. S: sensitivity; Sp: specificity; PPV: positive predictive value; NPV: negative preditive value.

A logistic regression model was constructed to evaluate independent predictors for C-CAN **(Table 4)**. After adjusting for age and sex, the only factor that remained significantly and independently associated with the development of complicated disease was a positive T2MR during the early period (OR: 36.5; 95%CI 1.7-779.2). The Hosmer Lemeshow goodness of fit test results indicate that the C-CAN model accurately reflected the data (p=0.02).

Table 4. Prediction of complicated candidemia. Multivariate analysis

VARIABLES	OR	95% CI	*p*
Age, years	0.94	0.8-1.2	0.54
Sex, male	0.75	0.02-16.1	0.75
Early positive blood culture	3.2	0.1-482.5	0.64
Early positive T2 MR	**36.5**	**1.7-779.6**	**0.02**
Early BDG	NE	NE	NE
Sepsis	2.5	0.1-74.8	0.60

NE Not evaluable

DISCUSSION

To our knowledge, this is the first report demonstrating that T2MR could potentially be used to monitor episodes of *Candida* BSI to predict outcomes. Compared with the traditional BCs and BDG assay, T2MR proved to be the

most effective method to recognize episodes of C-CAN in the early follow-up period, while being faster and easier to use.

Rapid diagnosis of metastatic complications is a challenging area for candidemia management.[15, 84] Several factors have been related to early development of complications in patients with candidemia, including *C. albicans* fungemia,[77, 94, 95] need for hemodialysis after candidemia,[79] and above all the persistence of positive BCs during follow-up period.[79, 80, 94, 96, 97] However, BCs have a low sensitivity for *Candida* species.[85] and are strongly influenced by antifungal therapy, thus further impairing their ability to identify patients with complicated candidemia. In the present study, only 4/9 (44.4%) patients with complicated candidemia had positive follow-up blood cultures. Therefore, new diagnostic methods that provide rapid and sensitive results for predicting complicated episodes of candidemia are needed.

T2MR is a new nano-diagnostic, fully automated technology that can directly and accurately detect molecular targets of *Candida* species within whole blood specimens, without the need for prior isolation.[98] This new technique has been studied and has demonstrated promising results for the diagnosis of candidemia;[55] however, to date, no reports have evaluated its role as a tool for early detection of complications in patients with previously diagnosed candidemia.

The findings of our study indicate that in comparison to BCs or the BDG assay, the performance of a follow-up T2MR in patients with a proven diagnosis of candidemia could better predict patients with complicated candidemia or poor

related prognosis, even if follow-up BCs are negative. In fact, a positive T2MR result performed within the first 5 days after the positivity of the index BC identifies a subgroup of patient with an almost 30-fold increase of developing a complication related to candidemia. Moreover, although the sensitivity was similar to that of BDG, the specificity of T2MR was about twice that of BDG, thus confirming the high rates of false positive BDG results in high risk populations.[85]

We believe that our results represent a significant step forward in the better management of candidemia. Follow-up T2MR could be useful to anticipate episodes of C-CAN and due to its ideal time to result[55], the results could be available early enough that diagnostic and management strategies could potentially modify patient outcomes. This is particularly true for patients with complicated candidemia where early timing remains a concern.

Although not directly evaluated in this study, the systematic use of T2MR during follow-up of candidemic patients could help clinicians to intervene early, and therefore improve the outcomes of patients with complicated disease. Early detection of a deep-seated candidiasis or of a metastatic complication could significantly change the clinical management of these patients. For example, an urgent source control of infection could be needed (especially in patients with infective endocarditis) or the selection of antifungal therapy and dosage could be influenced (switch to liposomal amphotericin B or fluconazole in case of ocular candidiasis or higher echinocandin dosage in case of infective endocarditis). Finally, since a positive T2MR during follow-up was associated

with a worse outcome, the decision regarding the admission ward (intensive care unit versus medical or surgical ward) could be modified.

This study has some limitations that should be assessed. First, our study conclusions are limited by the relatively small number of patients. Second, although systematic search for metastatic complications was recommended in all patients, only 24/30 (80%) patients had an echocardiogram performed, although in most cases, other complications were investigated according to clinical conditions. Third, long term outcome was not assessed and as the study was a proof of concept, T2MR results were not available for clinicians so we could not examine whether T2MR information would have changed patient management or outcomes. Strengths of our study include the fact that it is a prospective study performed in three large hospitals and that it represents important real life experience with T2MR in candidemic patients.

In conclusion, we found that a positive T2MR test performed in patients with proven candidemia may be a better marker of complicated infection than follow-up BC or the BDG assay. Given the difficulties in daily clinical practice of detecting episodes of complicated candidemia, our results support the use of T2MR a follow-up test in patients with candidemia, as well as the need for future research on its influence on duration of therapy and the type of antifungals used in candidemia management.

V. SEGUNDO OBJETIVO:

T2CandidaMR como predictor de la evolución en pacientes con sospecha de candidiasis invasiva que inician tratamiento antifúngico empírico

T2Candida MR as a predictor of outcome in patients with suspected invasive candidiasis starting empirical antifungal treatment

INTRODUCTION

Invasive candidiasis (IC) is the leading cause of fungal disease among hospitalized patients, and is associated with considerable morbidity, mortality and a high cost burden[99]. Early treatment in patients with proven candidemia is associated with reduced mortality [99] and consequently, this has produced an increase in early antifungal treatment in patients with risk factors of IC [65, 100-102]. However, IC is frequently not confirmed until after 3 to 5 days of empirical treatment and in that case, to stop or to continue antifungal agents is a real clinical challenge with influence in antifungal stewardship programs (median 11 days). [61]

In the last decade, strategies including biomarkers [β-D-glucan (BDG) or *Candida albicans* germ tube antibodies (CAGTA)], surveillance cultures, or predictive scores have been investigated not only for starting, [38, 103, 104] but also for discontinuing empirical antifungals [50, 105]. Our own data, and that of Nucci et al, suggests that patients with negative BDG and CAGTA results and with unconfirmed IC after 5-7 days of treatment, may be candidates for early discontinuation of antifungal agents. [50, 105].

T2MR is a new diagnostic method for diagnosis of candidemia, with an overall specificity of 99.4% and sensitivity of 91.1%.[55] To the best of our knowledge, its role on the management of patients with empirical antifungal agents due to suspected IC has not been evaluated. Therefore, we conducted a study to assess the potential role of T2MR alone and combined with serological biomarkers (BDG or CAGTA) and standard cultures for the early prediction of a

good/poor patient evolution in order to identify patients who would most benefit from discontinuation or maintenance of empirical antifungal therapy.

MATERIAL AND METHODS

Study population and setting

The second study was a prospective observational pilot study conducted in 4 hospitals located in Madrid, Spain. From January to June 2017, adult (>18 years) hospitalized patients who received systemic antifungal therapies for suspected IC were screened daily. The initiation of empiric antifungal treatment was based on the criteria specified in the IDSA guidelines [15]. Pregnant women, hematological patients, and those who had received >72 hours (h) of empiric antifungal therapy were not included. Patients were followed throughout the study period and for at least 1 month after they completed antifungal treatment.

Tests performed

Blood samples were collected by venepuncture to perform BDG, CAGTA and T2MR at the time the patient signed the informed consent (baseline, day 0) and then on day +2 and day +4. Only BDG and CAGTA results were reported to the attending clinicians, whereas T2MR results were provided only at the end of the study and thus, had no influence on the clinical management of the patients. Other routine microbiological (blood culture, catheter culture and culture of other

clinical samples) and radiological tests were also performed according to conventional clinical practice and local guidelines.

Definition of IC episode and Candida colonization

Invasive candidiasis included candidemia and deep seated candidiasis [74] which were defined as 1) the presence of *Candida* in blood in a patient with consistent clinical manifestations; 2) isolation of *Candida* species from normal sterile body fluids or peritoneal fluid obtained during surgery or by percutaneous aspiration or by drainage inserted for <24 h, respectively. Colonization was defined as the recovery of *Candida* species from non-sterile sites (urine, stool, drainage >24 h), independently of the presence of signs or symptoms attributable to the clinical suspicion of IC.

Definition of poor outcome episode

Poor outcome was defined as either diagnosis of IC and/or death within the first 7 days after starting empiric antifungal therapy, without another definitive identifiable cause. We decided to choose this definition because, from a clinical point of view, patients at risk of a poor outcome are purportedly those who could most benefit of the of an empirical antifungal treatment.

Data collection

Clinical and microbiological information was collected directly at the bedside by three investigators (AV, MM, FG). The data collected included age,

sex, risk factors for development of IC (*Candida* colonization, severity of illness, exposure to broad spectrum antibiotics, recent major surgery, particularly abdominal surgery, necrotizing pancreatitis, dialysis, parenteral nutrition, corticosteroids, and the use of central venous catheter), underlying disease, Charlson comorbidity index, McCabe score [89], clinical presentation, type of antifungal therapy and dosage, final diagnosis, reasons of ending treatment, length of hospital stay and overall mortality during hospitalization.

Laboratory procedures

BDG, CAGTA and T2MR samples were frozen at -20^{o}C until processed. All tests were performed twice weekly at the clinical laboratory of the coordinating center (Hospital General Universitario Gregorio Marañón).

Serological detection of BDG was performed using the Fungitell assay kit (Associates of Cape Cod) according to the manufacturer's instructions. Results were read and analyzed with a BioTek ELX808TM Microplate Reader and GEN5 Software (BioTek U.S., VT, USA) and considered positive when the values were ≥80 pg/mL.[50]

As for CAGTA assay, serum samples were processed according to the manufacturer's recommendations (Vircell Microbiologist S.L., Granada, Spain). Samples were considered positive above a cut-off ≥1/160.[106]

The T2MR (T2 Biosystems Inc.) was performed in EDTA whole-blood samples obtained from a peripheral vein and processed automatically following all the steps previously described by Mylonakis et al.[55] The technique detected

the five most frequent species involved in IC, coupled according to their resistance patterns:[15] *C. albicans* / *C. tropicalis*, *C. krusei* /*C. glabrata*, and *C. parapsilosis*. If the internal control was invalid and there were no positive T2MR signals, an "invalid" result was displayed, indicating that the specimen may have contained inhibitors that could have interfered with *Candida* detection.[55]

Data analysis

All analyses were performed using SPSS 18.0 (SPSS Inc., Chicago, IL, USA). For all calculations, a *p* value <0.05 was considered significant. Qualitative variables appear with their frequency distribution. Quantitative variables are expressed as the median and IQR. Groups were compared using the Fisher exact test for categorical variables and the Mann–Whitney test for continuous variables. A multivariate logistic regression model was used to assess predictors of poor outcome.

Performance characteristics (sensitivity, specificity, positive predictive value (PPV) and negative predictive value (NPV)) of the prediction rule of poor outcome were comprised of standard cultures, biomarkers and T2MR at different time-points. The goals of the diagnostic combinations procedures were to maximize the positive predictive value (identifying patients at high likelihood of poor outcome in whom antifungal should be maintained) while maintaining a high sensitivity (identifying a significant number of patients with poor outcome as positive).

Ethics

This study was approved by the Ethics Committee of Hospital General Universitario Gregorio Marañnon (number 2016-024) and the Spanish Agency for Medicines and Health Care Products. Written consent was deemed necessary.

RESULTS

Fifty-four consecutive patients anticipated to receive antifungal therapy for presumed IC were invited to participate in the study. Of these, 5 were not included in the final analysis for the following reasons: 3 (5.6%) invalid T2MR results, 1 (1.9%) proven infection caused by a *Candida* species not included in T2MR (secondary peritonitis due to *C. kefyr)* and 1 patient who did not sign the informed consent. The remaining 49 patients constitute the basis of our report.

Description of the groups

Table 5 summarizes the demographics and clinical characteristics of the 49 patients included. Most patients were male (65.3%) and the mean age was 64.7 years. Overall, 38 patients were admitted to ICU (77.6%) and 11 to other wards (14.3% medical and 8.2% surgical). The main indication for starting empiric antifungal therapy were severe sepsis or septic shock (69.4%) and central venous catheter in place (71.4%) or high risk gastrointestinal surgery

63.3%. Echinocandins accounted for 85.7% of the empirical therapy and the mean (±SD) duration of antifungal therapy was 9.5 days (SD 6.8 days).

Table 5. Comparison of demographic, clinical data, T2MR and biomarkers results between patients with good and poor outcome.

VARIABLES	**TOTAL (n=49)**	**Good outcome (n=35)**	**Poor outcome (n=14)**	***p***
Age, years, (mean ± SD)	64.7 ± 14.1	63.7 ± 14.8	67.2 ± 12.3	0.43
Sex, male	32 (65.3)	24 (68.6)	8 (57.1)	0.51
Hospital Ward				
ICU	38 (77.6)	27 (77.1)	11 (78.6)	1
Medical	7 (14.3)	5 (14.3)	2 (14.3)	1
Surgical	4 (8.2)	3 (8.6)	1 (7.1)	1
Underlying disease				
Gastrointestinal	23 (46.9)	16 (45.7)	7 (50.0)	1
Neoplastic	17 (34.7)	11 (31.4)	6 (42.9)	0.51
Cardiovascular	13 (26.5)	10 (28.6)	3 (21.4)	0.73
Chronic liver disease	9 (18.4)	7 (20.0)	2 (14.3)	1
Chronic renal failure	7 (14.3)	6 (17.1)	1 (7.1)	0.65
Solid organ transplantation	4 (8.2)	3 (8.6)	1 (7.1)	1
Charlson comorbidity index (mean ± SD)	3.0 ± 2.6	3.0 ± 2.6	3.0 ± 2.7	0.93
McCabe score				
Nonfatal disease	19 (38.8)	14 (40.0)	5 (35.7)	1
Ultimately fatal disease	26 (53.1)	21 (60.0)	5 (35.7)	0.20
Rapidly fatal disease	4 (8.2)	0 (0)	4 (28.6)	**0.005**
Reasons for empiric AF therapy				

Broad spectrum antibiotics	34 (69.4)	26 (74.3)	8 (57.1)	0.30
Central venous catheter	35 (71.4)	25 (71.4)	10 (71.4)	1
Severe sepsis or Septic shock	34 (69.4)	23 (65.7)	11 (78.6)	0.50
Surgery, previous 30 days	31 (63.3)	21 (60.0)	10 (71.4)	0.52
Total parenteral nutrition	18 (36.7)	14 (40.0)	4 (28.6)	0.52
Clinical evidence of intra-abdominal	21 (42.9)	13 (37.1)	8 (57.1)	0.22
infection	11 (22.4)	7 (20.0)	4 (28.6)	0.70
GI perforation or dehiscence	8 (16.3)	5 (14.3)	3 (21.4)	0.67
Need of haemodialysis				
Type of initial AF therapy				
Echinocandins	42 (85.7)	30 (85.7)	12 (85.7)	1
Azoles	6 (12.2)	4 (11.4)	2 (14.3)	1
Liposomal-AMB	1 (2.0)	1 (2.9)	0	1
***Candida* colonization (previous month)**	11(22.4)	10 (28.6)	1 (7.1)	0.14
Need for mechanical ventilation, previous 30 days	28 (57.1)	18 (51.4)	10 (71.4)	0.33
Days of AF before first study sample, mean ± SD	2.0 ± 1.0	2.0 ± 1.0	1.7 ± 1.3	0.31
Biomarkers results				
Positive CAGTA, baseline	11 (22.4)	8/35 (22.9)	3/14 (21.4)	1
Positive CAGTA, day + 2	10 (20.4)	8/35 (22.9)	2/13 (15.4)	0.70
Positive CAGTA, day + 4	7 (14.3)	5/34 (14.7)	2/9 (22.2)	0.62
Any positive CAGTA results for the total days (baseline, + 2 or + 4)	15 (30.6)	12 (34.3)	3 (21.4)	0.50
Positive BDG, baseline	25 (51.0)	17/35 (48.6)	8/14 (57.1)	0.75
Positive BDG, day + 2	19 (38.8)	13/35 (37.1)	6/12 (50.0)	0.51
Positive BDG, day + 4	21 (42.9)	17/34 (50)	4/9 (44.4)	1
Any positive BDG (baseline, + 2 or + 4)	32 (65.3)	22 (62.9)	10 (71.4)	0.74
Positive T2 MR, baseline	5 (10.2)	0/35 (0)	5/14 (35.7)	**<0.001**
Positive T2 MR, day + 2	2 (4.1)	2/35 (5.7)	1/13 (7.7)	1

Positive T2 MR, day + 4	3 (6.1)	1/34 (2.9)	2/9 (22.2)	0.10
Any positive T2MR result (baseline, +2 or +4)	8 (16.3)	2/35 (5.7)	6/14 (42.9)	**0.004**
Total days of AF therapy, mean ± SD	9.5 ± 6.8	8.4 ± 5.1	12.3 ± 9.7	**0.06**
Proven Invasive candidiasis	7 (14.3)	0 (0)	7 (50.0)	**<0.001**
In hospital mortality	17 (34.7)	8 (28.6)	9 (64.0)	**0.02**
Mortality within 7 days	7 (14.3)	0 (0)	7 (50.0)	**<0.001**

According to our definition, 35 patients (71.4%) had a good outcome and 14 (28.6%) had a poor outcome. Among poor outcome patients, 7 (50.0%) died within 7 days after starting empiric antifungal therapy and the other 7 (50.0%) had a diagnosis of deep seated candidiasis, one with candidemia. Overall, intra-abdominal infections accounted for 85.7% (6 out of 7) of deep seated candidiasis cases.

Details from these patients are provided in ***Supplementary material 1***. The *C. parapsilosis* that caused the fungemia of intra-abdominal origin was detected by the T2MR instrument 8 days before blood cultures became positive.

No differences were observed between the poor and good outcome groups with regard to age, sex, hospital ward, underlying disease, reason for empiric antifungal therapy, type of antifungal therapy, previous *Candida* colonization, and positive CAGTA or positive BDG assay **(Table 5).** However, compared to patients with good outcome, those with poor outcome had more rapidly fatal underlying disease according to McCabe scale (0 versus 28.6%,

p=<0.001) and received longer antifungal therapy, although such difference was not statistically significant (mean days 8.4 versus 12.3, p=0.06).

As for biomarkers, CAGTA and BDG could not differentiate both populations, since the results were positive in a significant number of patients with good outcome (baseline positive CAGTA: 8/35 [22.9%] and positive BDG: 17/35 patients [48.6%]). On the contrary, baseline T2MR was significantly more common in patients with poor outcome versus patients with good outcome (5/14 [35.7%] versus 0/35 [0.0%], p=0.001)**.** Interestingly, among patients with good clinical outcome, baseline T2MR was always negative. The T2MR result became positive on day +2 in 2 patients (5.7%), both had septic shock and multiple *Candida* colonization, but had no definitive diagnosis of IC, and had a good outcome.

After adjusting for age, sex, severity of underlying disease and clinical presentation, the multivariate analysis (data not shown) identified a positive T2MR performed at baseline as an independent predictor of poor outcome (OR 26.4- 95% CI 2.1-327.3, p=0.01).

Performance of T2MR, CAGTA, BDG and cultures, alone or in combination, to identify patients with poor outcome

Table 6 summarizes the sensitivity, specificity, and predictive values of the three techniques (BDG, CAGTA and T2MR) for identification of patients with poor outcome at different time-points (baseline, day +2 and day +4). For all three procedures, the results were better when tests were performed at

baseline. The best efficacy was achieved with T2MR, with a sensitivity of 35.7% (95% CI 13.9-64.3), a specificity of 100% (95% CI 87.6-100), a PPV of 100% (46.3-100) and a NPV of 79.6% (95% CI 64.2-89.6).

Table 6. Performance of diagnostic procedures for predicting POOR OUTCOME (analysis for patient)

	Sensitivity (95% CI)	**Specificity (95% CI)**	**PPV % (95% CI)**	**NPV % (95% CI)**
CAGTA,	21.4 (5.7-51.1)	77.1 (59.4-88.9)	27.2 (7.3-60.7)	71.0 (53.8-84.1)
CAGTA, day 2	15.3 (2.7-46.3)	77.1 (59.4-88.9)	20.0 (3.5-55.7)	71.0 (53.8-84.0)
CAGTA, day 4	22.2 (3.9-59.8)	85.3 (68.1-94.4)	28.5 (5.1-69.7)	80.5 (63.4-91.1)
Any positive CAGTA result	21.4 (5.7-51.1)	65.7 (47.7-80.3)	20.0 (5.3–48.6)	67.6 (49.3-82.0)
BDG, baseline	57.1 (29.6-81.1)	51.4 (34.2-68.2)	32.0 (15.7-53.5)	75.0 (52.9-89.4)
BDG, day 2	50.0 (22.2-77.7)	62.9 (44.9-78.0)	31.5 (13.5-56.5)	78.5 (58.5-90.9)
BDG, day 4	44.4 (15.3-77.3)	50.0 (32.7-67.2)	19.0 (6.2-42.5)	77.2 (54.1-91.3)
Any positive BDG result	71.4 (42.0-90.4)	37.1 (22.0-55.1)	31.3 (16.7-50.1)	76.4 (49.8-92.1)
T2 MR, baseline	35.7 (13.9-64.3)	100 (87.6-100)	100 (46.3-100)	79.6 (64.2-89.6)
T2 MR, day 2	8.3 (0.4-40.2)	94.2 (79.4-99.0)	33.3 (1.7-87.4)	75.0 (59.3-86.2)
T2 MR, day 4	20.0 (3.5-55.7)	97.1 (82.9-99.8)	66.7 (12.5-98.2)	80.5 (64.6-90.6)
Any positive T2MR result	42.8 (18.8-70.4)	94.2 (79.4-99.0)	75.0 (35.5- 95.5)	80.4 (64.4-90.6)

CI Confidence Interval; **PPV** Positive Predictive Value; **NPV** Negative Predictive Value

When we analyzed the efficacy of the combination of different biomarkers with standard cultures **(Table 7),** the best results were obtained with baseline T2MR combined with standard cultures.

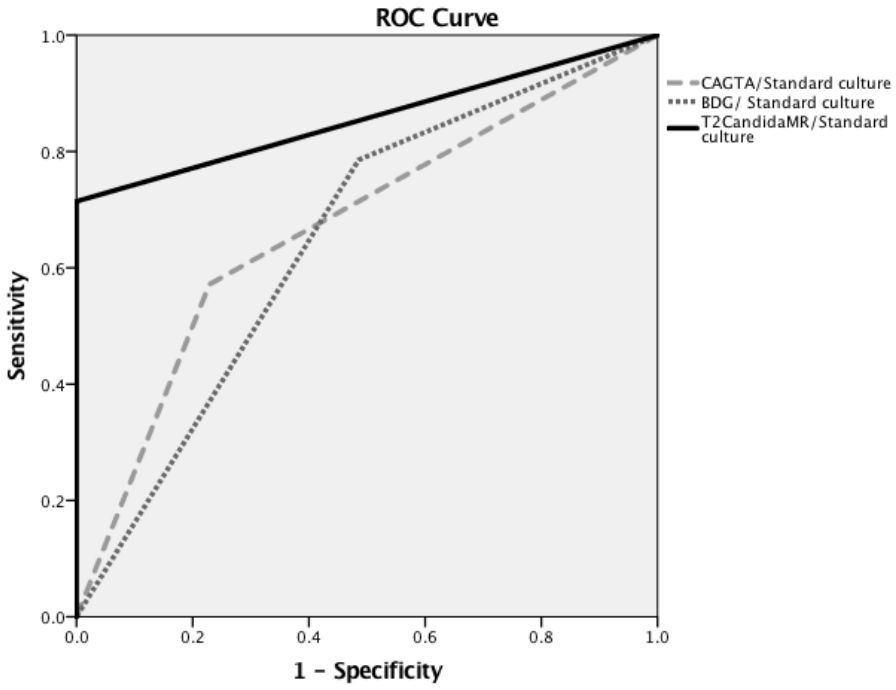
Table 7. Analysis of the efficacy of the combination of T2MR, biomarkers and cultures

	Sensitivity% (95% CI)	Specificity (95% CI)	PPV % (95% CI)	NPV % (95% CI)
CAGTA/BDG				
Baseline	71.4 (42.0-90.4)	10.0 (24.3-57.8)	32.3 (17.3-51.5)	77.8 (51.9-92.6)
Day 2	61.5 (32.2-84.8)	48.6 (31.7-65.7)	30.7 (15.1-51.9)	77.3 (54.2-91.3)
Day 4	66.6 (30.9-90.9)	47.1 (30.1-64.6)	25.0 (10.6-47.1)	84.2 (59.5-95.8)
Day 0,2,4	85.7 (56.1- 97.4)	22.8 (11.0-40.5)	30.7 (17.5-47.7)	80.0 (44.2-96.4)
CAGTA/T2MR				
Baseline	57.1 (29.6-81.1)	77.1 (59.4-88.9)	50.0 (25.5- 74-	81.8 (63.9-92.3)
Day 2	23.0 (6.1-54.0)	74.2 (56.4-86.9)	4)	72.2 (54.5-85.2)
Day 4	33.3 (9.0-69.0)	82.5 (64.8-92.6)	25.0 (6.6-57.9)	82.3 (64.8-92.6)
Day 0,2,4	72.7 (39.3- 92.6)	68.4 (51.2-81.9)	33.3 (9.0 -69.1)	89.6 (71.5-97.2)
			40.0 (20.0-63.6)	
BDG/T2MR				
Baseline	64.2 (35.6-86.0)	51.4 (34.3-68.3)	34.6 (17.9-55.6)	78.3 (55.8-91.7)
Day 2	58.3 (28.6-83.5)	57.1 (39.5-73.3)	31.8 (14.7-54.9)	80.0 (58.7-92.4)
Day 4	55.5 (22.6-84.6)	47.1 (30.1-64.6)	21.7 (8.2-44.3)	80.0 (55.8-93.3)
Day 0,2,4	85.7 (56.1- 97.4)	37.1 (18.7-45.6)	35.3 (20.3-53.5)	86.7 (58.4-97.6)
CAGTA/Standard cultures				
Baseline	57.1 (29.6-81.1)	77.1 (59.4-88.9)	50.0 (25.5-74.5)	81.8 (63.9-92.4)
Day 2	50.0 (24.0-75.9)	77.1 (59.4-88.9)	46.6 (22.3-72.6)	79.4 (61.5-90.6)
Day 4	50.0 (24.0-75.9)	85.7 (68.9-94.6)	58.3 (28.6-83.5)	81.1 (64.3-91.4)
Day 0,2,4	57.1 (29.6-81.1)	65.7 (47.7-80.3)	40.0 (19.9-63.6)	79.3 (59.7-91.3)
BDG/Standard cultures				
Baseline	78.5 (48.8-94.2)	51.4 (34.2-68.3)	39.3 (22.1-59.2)	85.8 (62.6-96.3)

Day 2	71.4 (42.0-90.4)	62.8 (44.9-78.0)	43.5 (23.9-65.1)	84.6 (64.2-94.9)
Day 4	50.0 (22.3-77.7)	51.4 (34.3-62.3)	26.1 (11.8-48.6)	75.0 (52.9-89.4)
Day 0,2,4	85.7 (56.1-97.4)	37.1 (22.0-55.1)	35.3 (20.3-53.6)	86.7 (58.4-97.6)
T2Mr/Standard cultures				
Baseline	71.4 (42.0-90.4)	100 (87.6-100)	100 (65.5-100)	89.7 (74.8-96.6)
Day 2	57.1 (29.6-81.2)	94.2 (79.4-99.0)	80.0 (44.2-96.4)	84.6 (68.8-93.5)
Day 4	50.0 (24.0-75.9)	97.1 (83.3-99.8)	87.5 (46.7-99.4)	82.9 (67.4-92.3)
Day 0,2,4	71.4 (42.0-90.4)	94.2 (79.4-99.0)	83.3 (50.9-97.1)	89.2 (73.6-96.5)

Figure 1 depicts the ROC curves for these combinations at baseline. The combination of baseline T2MR with standard cultures showed the highest AUC (0.85), in comparison with that of CAGTA/standard culture (0.67) and BDG/standard culture (0.65) combinations.

FIGURE 1. ROC curves showing the efficacy of baseline CAGTA, BDG or T2MR combined with standard cultures for the diagnosis of patients with poor outcome.



DISCUSSION

In the second paper of my thesis including a cohort of hospitalized patients at high risk of IC, we found that a positive early T2MR was independently associated with a poor outcome, defined as ulterior demonstration of IC or unexplained death. In fact, baseline T2MR was always negative in patients with good outcome, so, purportedly, it may be a marker for early discontinuation of empirical antifungals.

Clinical suspicion of IC is common in critically ill patients, and, given the limitations of blood cultures for the diagnosis of deep-seated candidiasis,[85, 107-110] patients with suspected IC often receive empiric antifungal therapy for 10-14 days.[61] Different studies have demonstrated that the incidence of IC in these populations is relatively low,[111, 112] very similar to the 14% found in our study. Therefore, many patients may receive unnecessary antifungals with a negative impact on resistance rates,[113] adverse effects, and health care costs.[101, 114]

Although the utility of empiric antifungal therapy in high risk ICU patients has been recently questioned,[112] the fact is that it remains a common practice both inside and outside ICUs. Accordingly, there is a clear need to differentiate patients who could really benefit from antifungal therapy from patients in whom therapy could be stopped early.[115-117] Standard biomarkers and cultures require at least 5-7 days and the experience with early withdrawal of therapy is very limited.[50, 105]

T2MR is a new fully automated nano-diagnostic technology, that can detect molecular targets of *Candida* species within whole blood specimens, without the need for prior isolation,[98] providing results within 4 hours. These early results could prove essential for stopping unnecessary treatments, thus we evaluated its potential role as a marker for good prognosis in patients in whom empiric antifungal therapy had been started.

Notably, in the second study, we found that a positive T2MR in a sample obtained within the first 72 hours of empiric therapy (baseline) had a specificity of 100% for identifying poor outcome patients. Moreover, our study

demonstrates that when combined with standard cultures, the sensitivity reached 71.4%, while PPV remained 100%. Considering these results, a possible recommendation would be to consider stopping empirical antifungal therapy in patients with negative baseline T2MR and with negative cultures, and to maintain a full course in those with a positive test. With this approach, antifungal therapy could have been drastically shortened in 33 out the 49 high risk patients included in the present study (67.3%).

There are potential limitations of our study. First, in our study all patients had received a mean of two days of antifungal therapy before samples were obtained, and results of the T2MR were not transmitted to the clinician. Accordingly, a prospective comparative study should be done to evaluate the safety of our proposed approach to empirical antifungal therapy. Until such study is available, experts in the field should make decisions. Second, since we excluded from the analysis one patient with *C. kefyr* peritonitis (not detectable by T2MR), the diagnostic value of the T2MR could have been overestimated. Third, because T2MR panel include only five species of *Candida*, we did not consider in our analysis the possibility to have T2MR negative patients that are infected by other *Candida* species. Consequently, despite positive T2MR patients are candidates to be poor outcome patients, negative baseline T2MR patients need a negative culture confirmation in order to consider an early stopping of empirical antifungal therapy. Fourth, no patient was subjected to autopsy examination. Consequently, we cannot confirm that patients who died in the first 7 days actually had an IC. On the other hand, strengths of our proof

of concept study include its prospective nature and the fact that it was derived from a cohort of patients in four large academic medical centers, thus results may be generalizable to other settings.

In conclusion, in our cohort of patients with a presumed diagnosis of IC, T2MR, in combination with standard cultures, demonstrated a high discriminative ability for identifying patients with high risk of dying or developing IC. Taken in the context of other clinical and microbiological tests, this new diagnostic tool may be of significant utility to identify patients who may really benefit from antifungal therapy. Future studies designed to evaluate the impact of our findings on diagnosis, treatment, outcome and healthcare cost are warranted.

VI. TERCER OBJETIVO:

Eficacia de un paquete de medidas basado en una "check-list" sobre el manejo y la evolución clínica de los pacientes con candidemia

Efficacy of a "check list" intervention bundle on the clinical outcome of patients with Candida bloodstream infections

INTRODUCTION

Candida bloodstream infection (BSI) is a life-threating disease associated with significant morbidity, mortality and high healthcare costs. Complications are frequent [79, 80], and mortality ranges between 20% and 45% [11, 81, 83]

Prompt diagnosis, early administration of appropriate antifungal therapy and adequate source control of infection have been shown to be key factors that improve the prognosis of patients with candidemia [13, 15, 36, 63, 118]. Infectious disease (ID) consultations providing diagnostic and therapeutic bedside recommendations as a part of antifungal stewardship programs (ASP), has also been demonstrated to be associated with a better prognosis of candidemic patients [102, 119, 120].

However, the recommendations provided by the ID consultants in such studies were not structured according to a bundle approach, and specific interventions were not always completed in time.

Our hypothesis was that the systematic implementation of structured recommendations aimed at enhancing adherence to evidence-based indicators could improve both diagnostic procedures and antifungal therapy, and eventually the outcome of patients with candidemia. Therefore, the aim of this prospective study was to evaluate the all cause 14-day and 30-day mortality in candidemic patients before and after the implementation of a bedside check list care bundle to our antifungal stewardship program.

MATERIALS AND METHODS

Study setting

This study was performed at the Hospital Universitario General Gregorio Marañón, a 1,550-bed tertiary care institution in Madrid, with a full range of clinical services attending a population of approximately 715,000 inhabitants.

Study population

All consecutive adult patients (>18 years of age) with at least 1 episode of candidemia diagnosed at our hospital over the period (May 2014 to May 2017) were eligible for the study. Exclusion criteria were: patients with a life expectancy of<72 hours or those who were transferred to another healthcare facility

Study design

We performed a, pre-post quasi-experimental study. During the *pre-intervention period* (May 2014-September 2015) a non-mandatory ASP was implemented at our hospital [102] and patients with candidemia were visited by an ID specialist as soon as possible who provided diagnostic and therapeutic advice according to standard of care.

During the *post-intervention* period (October 2015-May2017), patients were managed according to a candidemia care bundle. Briefly, two ID physicians with specific expertise in mycology and antifungal therapy followed all candidemic patients and promoted adherence with bundle endpoints

providing clear and structured recommendations that were written in the medical history of the patients.

Patients were followed-up until 30 days from *Candida* BSI onset or until death.

Intervention

The care bundle for candidemia was created identifying “quality indicators” and consisted of six recommendations provided in a structured form and checked with a list. Bundle endpoints included: 1) early (<72 h) adequate antifungal therapy, 2) early (<72 h) source control, if necessary 3) follow-up blood cultures, 4) ophthalmologic examination, 5) echocardiogram, and a 6) adequate duration of therapy according to complexity of the infection. Additionally, we also included a standardized checklist to gather bundle compliance data and to periodically check the clinical condition of the patients, microbiological culture results and compliance to other measures that are generally accepted clinical practice, such as drug selection and drug dosing according to hepatic and renal function, drug-drug interactions, drug de-escalations **(Table 8).**

The study was approved by the ethics committee of the Hospital General Universitario Gregorio Marañon of Madrid and informed consent was asked to eligible patients.

Table 8. Candidemia bundle check list

DAY 0	**CHECK-LIST**
Check for sepsis and septic shock	
Presence of ocular symptoms	
Presence of cardiac murmur or intravascular device	
Previous azole use	
Drug-drug interaction	
Reviewing the previous microbiological cultures	
Choose the adequate antifungal drug according to clinical condition and previous cultures	
Check for adequate antifungal dosage according to weight, renal and hepatic function	
Request all necessary microbiological and radiological tests.	
Check for the number of CVC and peripheral catheters, as their status. Support all devices withdrawal when unnecessary.	
If necessary, CVC withdrawal and adequate control of other sources.	
DAY + 1	
Microbiological adjustment according to E-test and Maldi-tof results.	
Performance of follow-up blood cultures.	
Request echocardiography.	
Request ophalmoscopy.	
Request central venous echography if a clinical suspicion of thrombophlebitis is present.	
DAY +3	
Check for definitive antifungal susceptibility testing.	
Check if antifungal serum concentration is adequate, if clinically necessary.	
Check for negativity of previous follow-up blood cultures. If positive, request new blood cultures sets.	
Check for results of all previous microbiological cultures.	
Check for adequate source control of the infections.	
DAY +5	
Check for toxicity, drug-drug interactions and renal and hepatic functions.	

Check for negativity of previous follow-up blood cultures. If positive, request new blood cultures sets.	
If possible, step-down therapy.	
DAY +7	
Check for ophthalmoscopy and echocardiography results.	
Check for negativity of previous follow-up blood cultures. If positive request new blood cultures sets.	
DAY +14	
Check for all microbiological cultures, ophthalmoscopy and echocardiogram results.	
Check for renal and hepatic function.	
Establish length of antifungal therapy and program outpatient visit at 6 months.	

Endpoints

Considering that among patients with candidemia, 30 day mortality is mainly related to the underlying disease of the patients [13] and assuming that an adequate management of candidemia could have an impact on 14-day related mortality, the main outcome of our study was 14-day all-cause mortality. As secondary outcome, we considered the adherence to all the quality indicators of *Candida* bundle and 30-day mortality rate.

Definitions

An episode of candidemia was defined as a patient that had at least 1 peripheral blood culture positive for *Candida* species. Sepsis, septic shock, and Pitt's bacteraemia score were defined according to standard international criteria [90, 91]

As for the source of infection, an episode of candidemia was considered as catheter-related if 1) the catheter tip culture was positive with the same *Candida* species, 2) there was evidence of exit site catheter exudate with the same *Candida* species or 3) the differential time to positivity of BCs obtained from the catheter and peripheral veins was greater than or equal to 2 hours [92].

The urinary tract was considered to be the portal of entry in patients with urologic predisposing conditions (i.e. manipulation or obstruction of the urinary tract) and evidence of urinary tract infection caused by the same species of *Candida*.

The abdomen was considered to be the origin of the candidemia when a patient had evidence of abdominal infection and 1) a positive culture from the intra-abdominal space was obtained during surgery or by needle aspiration and/or 2) no other apparent sources of candidemia were detected. When a source of candidemia could not be identified, candidemia was defined as "primary".

Patients were considered to have *Candida* septic metastasis when an infection due to the same Candida species occurred in a site that was distant from the source of the candidemia. In cases in which no culture was available, the distant infection had to be temporally related with the fungemia and with no alternative cause explaining the clinical condition.

Infective endocarditis was diagnosed according to "the Duke criteria" [93]; ocular candidiasis was classified based on previous criteria; [79, 121]; septic

thrombophlebitis required the presence of venous thrombosis, confirmed by imaging techniques, in the setting of persistent candidemia. [15].

Early antifungal treatment was defined as adequate if a recommended dose of an antifungal drug was administered within 72 hours after candidemia onset and it was found to be effective by in-vitro susceptibility testing. Early adequate source control was defined as removal of the indwelling catheter or surgical drainage of deep infection within 72 hours after the index blood sample was drawn. For patients with candidemia without metastatic infection, duration of antifungal therapy was considered adequate when it lengths at least 14 days form the first negative blood cultures. For patients with candidemia with metastatic infections (i.e. ocular candidiasis, lung metastasis) and or other complications (i.e. thrombophlebitis), duration of treatment was considered adequate when it lengths at least 4-6 weeks or, even longer in case of infective endocarditis.

Data collection

Data were prospectively recorded on a standardized case report form that included demographics; comorbidities; predisposing risk factors within the preceding 30 days; clinical severity according to Pitt score (ref); source of the infection; adherence to *Candida* bundle; clinical management of the patients including antifungal choice, length of therapy and adequate source control of the infection; and all-cause mortality.

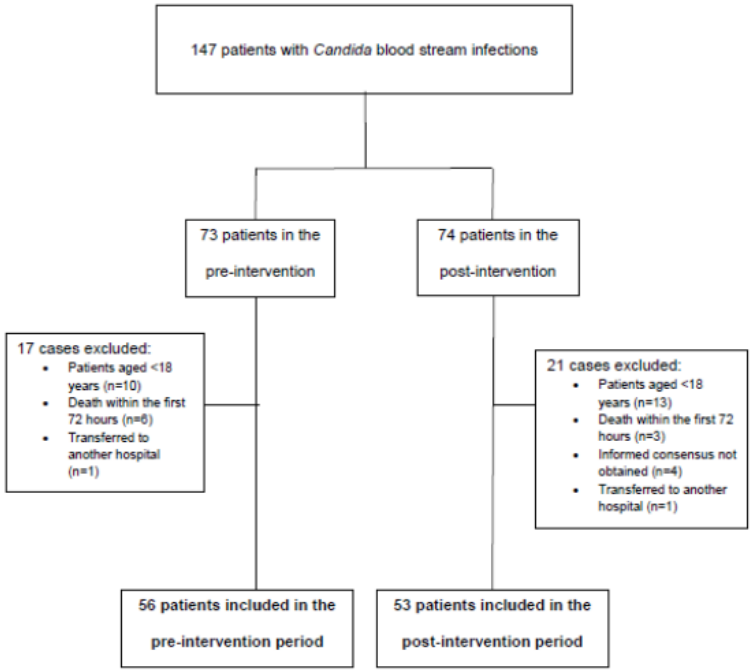
Statistical analysis

Descriptive statistics were used to summarize the data. Quantitative variables are reported as median and interquartile range (IQR), and categorical variables as counts (%). The chi-square test or Fisher exact tests were used to compare the distribution of categorical variables, including the clinical characteristics of pre and post interventional period and the association between individual risk factors and mortality rate. The t test or Mann-Whitney test was used to compare quantitative variables. Statistical significance was set at $p<0.05$. The Kaplan-Meier curve was constructed to show the relationship between intervention strategy and 14-day survival. The statistical analyses were performed using Microsoft SPSS PC+, version 15.0 (SPSS, Chicago, Illinois, USA).

RESULTS

The flowchart study is shown in **Figure 2** Overall, 147 patients were diagnosed with an episode of candidemia, 73 in pre-intervention and 74 in post-intervention period, respectively. In the pre-intervention period, 10 patients were excluded because aged <18 years and six died within the first 72 hours and one patient was transferred to other hospital. In the post-interventional 13 patients were excluded because aged <18 years, four because of declining to participate, three died within the first 72 hours and one was transferred to other healthcare facility.

Figure 2. Flow chart of patients included in the study



The demographics and clinical features of the 109 patients of the study populations are shown in **Table 9**. Mean age was 67.2 years and 73/109 patients were males (67.0%). The most common underlying condition was solid tumor (60/109; [55.0%]) followed by gastrointestinal disease (44/109, [40.7%]). A CVC was in place in 86/109 patients (78.9%) and 70/109 (64.2%) were receiving total parenteral nutrition at the time of their episode. The most

prevalent source of infection was the CVC (65/109; [59.6%]) followed by the intra-abdominal origin (18/109; [16.5%]). Only 13/109 patients (11.9%) had a primary infection.

Table 9. Comparison of patients with candidemia who were managed according to comprehensive Care Bundle OR not (controls)

Variables	Total population (n=109)	Pre-Intervention group (n=56)	Post-Intervention group (n=53)	*p* Value*
Age (years), mean ± SD	67.2 ± 13.9	66.6 ± 13.5	67.8 ± 14.4	0.67
Male, n (%)	73 (67.0)	37 (66.1)	36 (67.9)	1
Department, n (%)				
Medical ward	38 (34.9)	17 (30.4)	21 (39.6)	0.32
Surgical ward	36 (33.0)	23 (41.1)	13 (24.5)	0.07
ICU stay	23 (21.1)	10 (17.9)	13 (24.5)	0.48
Oncology-hematology ward	12 (11.0)	6 (10.7)	6 (11.3)	1
Underlying disease, n (%)				
Solid tumor	60 (55.0)	31 (55.4)	29 (54.7)	1
Gastrointestinal disease	44 (40.7)	25 (45.5)	19 (35.8)	0.33
Diabetes mellitus	26 (23.9)	12 (21.4)	14 (26.4)	0.65
Neurologic disease	26 (23.9)	12 (21.4)	14 (26.4)	0.65
Cardiovascular disease	25 (22.9)	13 (23.2)	12 (22.6)	1
Liver disease	15 (13.9)	10 (18.2)	5 (9.4)	0.26
Hematological malignancy	6 (5.5)	2 (3.6)	4 (7.5)	0.43
Charlson comorbidity index, mean ± SD	3.6 ± 2.5	3.6 ± 2.6	3.6 ± 2.5	0.93

Risk factor, no (%)				
Central venous catheter				
Total parenteral nutrition	86 (78.9)	48 (85.7)	38 (71.7)	0.10
Previous abdominal	70 (64.2)	35 (62.5)	35 (66.0)	0.84
surgery	45 (41.3)	26 (46.4)	19 (35.8)	0.33
Corticosteroids	34 (31.2)	16 (28.6)	18 (34.0)	0.67
Previous antifungals	31 (28.4)	15 (26.8)	16 (30.2)	0.83
Neutropenia	8 (7.3)	5 (8.9)	3 (5.7)	0.71
Immunosuppressivion	8 (7.3)	3 (5.4)	5 (9.4)	0.48
Pitt score, median (IQR)	0 (0-2)	0 (0-2)	0 (0-2)	0.64
Time between hospitalization and candidemia onset, median (IQR)	23.0 (9.0-39.0)	22.0 (6.5-38.5)	23.0 (12.0-45.0)	0.55
Clinical manifestation, n (%)				
Sepsis	40 (36.7)	23 (41.1)	17 (32.1)	0.42
Severe sepsis	31 (28.4)	20 (35.7)	11 (20.8)	0.09
Septic shock	12 (11.0)	4 (7.1)	8 (15.1)	0.22
***Candida* species, n (%)**				
C. albicans	56 (51.4)	25 (44.6)	31 (58.5)	0.18
C. parapsilosis	27 (24.8)	14 (25.0)	13 (24.5)	1
C. glabrata	15 (13.2)	8 (14.3)	7 (13.2)	1
C. krusei	6 (5.5)	4 (7.1)	2 (3.8)	0.67
C. tropicalis	4 (3.7)	2 (3.6)	2 (3.8)	1
Other *Candida* species[a]	3 (2.8)	3 (5.4)	0	0.24
Source, n (%)				
Central venous catheter	65 (59.6)	33 (58.9)	32 (60.4)	1
Intra-abdominal	18 (16.5)	12 (21.4)	6 (11.3)	0.20
Primary	13 (11.9)	6 (10.7)	7 (13.2)	0.77

Urinary tract	7 (6.4)	4 (7.1)	3 (5.7)	1
Others [b]	6 (5.5)	1 (1.8)	5 (9.4)	0.10
Initial antifungal therapy, n(%)				
Fluconazole	74 (67.9)	38 (67.8)	36 (67.9)	1
Echinocandins	29 (26.6)	15 (26.7)	14 (26.4)	1
Liposomal amphotericin B	6 (5.5)	3 (5.4)	3 (5.7)	1
ICU admission, n(%)	10 (9.2)	5 (8.9)	5 (9.4)	1
Length of hospital stay (days) , median (IQR)	50.0 (31.0-88.5)	46.5 (22-86.7)	51 (37-97.5)	0.96

[a] Other Candida species include: 1 *C. lusitaniae*, 1 *C. dublinensis* and 1 *C. incospicua*

[b] Other sources include: 1 chorioamnionitis, 2 peripheral catather. 2 infective endocarditis, 1 infection from prosthesis

ICU, intensive care units

*P values < 0.05 are shown in bold

Comparison between *pre* and *post* intervention period

The main demographic characteristic and risk factors for candidemia in the pre and post intervention period are compared in **Table 9**. Both populations were similar and no statistically significant differences were detected regarding demographics, underlying diseases, risk factors for candidemia, severity of disease, *Candida* species and source of infection. During the pre-intervention period, ID physicians visited patients in 50/56 cases (89.2%) whereas 53/53 patients (100%) were visited in the post-intervention period (p=0.71)

Overall, compliance with *Candida* bundle significantly improved between pre (27/56, [48.2%]) and post-intervention (43/53, [81.1%]; p=0.01) period (**Table 10)**. Individual bundle components significantly improved in the post-intervention period were early adequate antifungal therapy (47/56, [83.9%] vs 51/53, [96.2%], p=0.05), early adequate source control of the infection (37/56, [82.2%] vs 41/53, [97.6%], p=0.03) and appropriate duration of therapy (27/56, [48.2%] vs 43/53, [81.1%], p=0.01). Moreover, adherence to follow-up blood cultures, ophthalmologic examination and echocardiography improved in the post-intervention period, but the difference was not statistically significant.

Table10. Compliance and impact of a comprehensive Care Bundle on candidemia

	Intervention group (n=56)	**Control Group (n=53)**	***p* Value***
All bundle elements successfully completed	27 (48.2)	43 (81.1)	**0.01**
Early adequate source control of infection	37 (82.2)	41 (97.6)	**0.03**
Early adequate antifungal therapy	47 (83.9)	51 (96.2)	**0.05**
At least one complication detected	10 (20.8)	19 (38.0)	0.08
Blood cultures every 48 hours until negative	50 (89.3)	51 (96.2)	0.27
Persistent candidemia	15/51 (29.4)	8/51 (16.0)	0.15
Ophthalmologic examination performed	47 (83.9)	49 (92.5)	0.23
Ocular candidiasis	5/47 (10.6)	10/49 (20.4)	0.26
Echocardiograms performed	47 (83.9)	48 (90.6)	
Trans-Thoracic	22 (46.8)	18 (37.5)	0.34
Trans-Esophageal	25 (53.2)	30 (62.5)	
Infective endocarditis	0/46 (0)	2/48 (4.2)	0.49
Other complications			
Thrombophlebitis	4/9 (44.4)	5/11 (45.5)	1
Spread to other organs	1/56 (1.8)	7/53 (13.2)	0.03
Appropriate duration of therapy	45 (80.4)	51 (96.2)	**0.01**

*P values < 0.05 are shown in bold

Analysis of all cause of mortality at 14 and 30 days

Overall, all-cause mortality at 14 and 30 days was 14.9% (16/109) and 27.5% (30/109). Variables associated with 14-day and 30-day mortality in the univariate analyses are summarized in the **Table 11** and **Table 12**. Being managed according to candidemia bundle had a favorable impact on 14-day mortality (50/93 [53.8%] versus 3/16 [18.8%], p=0.01) but not on 30-day mortality rate (41/79 [51.9%] versus 12/30 [40%], p=0.29). However, after controlling for baseline-characteristics, clinical presentation of candidemia and source of infection, being managed according to candidemia bundle remained the only variable independently associated with a decreased all-cause mortality at both 14 (HR 0.08, 95%CI 0.01-0.45, p=0.02) and 30-day (HR 0.40, 95%CI 0.18-0.89, p=0.02) (**Table 13**).

Table 11 Univariate analysis of variables associated with 14-day mortality

Variables	Alive (n=93)	Dead (n=16)	*p* Value*
Age (years), mean ± SD	66.3 ± 13.6	72.1 ± 15.3	0.13
Male, n (%)	62 (66.7)	11 (68.8)	1
Department, n (%)			
Surgical ward	34 (36.6)	2 (12.5)	0.08
Medical ward	29 (31.2)	9 (56.3)	0.08
ICU stay	21 (22.6)	2 (12.5)	0.51
Oncology-hematology ward	9 (9.7)	3 (18.8)	0.37

Underlying disease, n (%)			
Solid tumor	52 (55.9)	8 (50.0)	0.78
Gastrointestinal disease	38 (41.3)	6 (37.5)	1
Diabetes mellitus	23 (24.7)	3 (18.8)	0.75
Neurologic disease	23 (24.7)	3 (18.8)	0.75
Cardiovascular disease	19 (20.4)	6 (37.5)	0.19
Liver disease	13 (14.1)	2 (12.5)	1
Hematological malignancy	4 (4.3)	2 (12.5)	0.21
Charlson comorbidity index	3.6 ± 2.6	3.4 ± 2.4	0.75
Risk factor, no (%)			
Central venous catheter	75 (80.6)	11 (68.8)	0.32
Total parenteral nutrition	62 (66.7)	8 (50.0)	0.26
Previous abdominal surgery	40 (43.0)	5 (31.3)	0.42
Corticosteroids	28 (30.1)	6 (37.5)	0.56
Previous antifungals	24 (25.8)	7 (43.8)	0.23
Neutropenia	6 (6.5)	2 (12.5)	0.33
Immunosuppressive therapy	7 (7.5)	1 (6.3)	1
Pitt score, median (IQR)	0 (0-2)	0 (0-1)	0.46
Clinical manifestation, n (%)			
Sepsis	35 (37.6)	5 (31.3)	0.78
Severe sepsis	28 (30.1)	3 (18.8)	0.54
Septic shock	8 (8.6)	4 (25.0)	0.07
***Candida* species, n (%)**			
C. albicans	47 (50.5)	9 (56.3)	0.78
C. parapsilosis	24 (25.8)	3 (18.8)	0.75
C. glabrata	13 (14.0)	2 (12.5)	1
C. krusei	4 (4.3)	2 (12.5)	0.21
C. tropicalis	4 (4.3)	0	1
Other *Candida* species	3 (3.2)	0	1

Source, n (%)			
Central venous catheter	60 (64.5)	5 (31.3)	**0.02**
Intra-abdominal	14 (15.1)	4 (25.0)	0.29
Primary	8 (8.6)	5 (31.3)	**0.02**
Urinary tract	6 (6.5)	1 (6.3)	1
Other sources[b]	5 (5.4)	1 (6.3)	
Initial antifungal therapy, n(%)			
Fluconazole	63 (67.7)	11 (68.8)	1
Echinocandins	25 (26.8)	4 (25.0)	1
Liposomal amphotericin B	5 (5.3)	1 (6.3)	1
Early adequate antifungal therapy, n(%)	85 (91.4)	13 (81.3)	0.20
Early adequate source control of infection, n(%)	73 (93.6)	5 (55.6)	**0.006**
Persistent candidemia, n(%)	21 (23.1)	2 (20.0)	1
Ocular candidiasis, n(%)	15 (16.9)	0	0.59
Infective endocarditis, n(%)	2 (2.3)	0	1
ICU admission due to candidemia, n(%)	6 (6.5)	4 (25.0)	**0.04**
Intervention period, n(%)	50 (53.8)	3 (18.8)	**0.01**
All bundle elements successfully completed, n(%)	65 (69.9)	5 (31.3)	**0.004**

[a] Other Candida species include: 1 *C. lusitaniae*, 1 *C. dublinensis* and 1 *C. incospicua*

[b] Other sources include: 1 chorioamnionitis, 2 peripheral catather. 2 infective endocarditis, 1 infection from prosthesis

ICU, intensive care units

*P values < 0.05 are shown in bold

Table 12 Univariate analysis of variables associated with 30-day mortality

Variables	Alive (n=79, %)	Dead (n=30, %)	*p* Value*
Age (years), mean ± SD	66.5 ± 14.1	69.1 ± 13.7	0.40
Male, n (%)	26 (32.9)	10 (33.3)	1
Department, n (%)			
Surgical ward	33 (41.8)	3 (10.0)	**0.001**
Medical ward	25 (31.6)	13 (43.3)	0.26
ICU stay	14 (17.7)	9 (30.0)	0.19
Oncology-hematology ward	7 (8.9)	5 (16.7)	0.30
Underlying disease, n (%)			
Solid tumor	44 (55.7)	16 (53.3)	0.83
Gastrointestinal disease	35 (44.9)	9 (30.0)	0.19
Diabetes mellitus	21 (26.6)	5 (16.7)	0.32
Neurologic disease	20 (25.3)	6 (20.0)	0.62
Cardiovascular disease	16 (20.3)	9 (30.0)	0.31
Liver disease	12 (15.4)	3 (10.0)	0.55
Hematological malignancy	2 (2.5)	4 (13.3)	**0.04**
Charlson comorbidity index, median (IQR)	3 (2-6)	3 (2-6)	0.53
Risk factor, no (%)			
Central venous catheter	62 (78.5)	24 (80.0)	1
Total parenteral nutrition	50 (63.3)	20 (66.7)	0.82
Previous abdominal surgery	36 (45.6)	9 (30.0)	0.19
Corticosteroids	21 (26.6)	13 (43.3)	0.10
Previous antifungals	20 (25.3)	11 (36.7)	0.24
Neutropenia	3 (3.8)	5 (16.7)	**0.03**
Immunosuppressive therapy	5 (6.3)	3 (10.0)	0.68
PITT score, median (IQR)	0 (0-2)	1 (0-3.25)	0.05

Clinical manifestation, n (%)			
Sepsis	32 (40.5)	8 (26.7)	0.26
Severe sepsis	24 (30.4)	7 (23.3)	0.63
Septic shock	4 (5.1)	8 (26.7)	**0.003**
***Candida* species, n (%)**			
C. albicans	39 (49.4)	17 (56.7)	0.52
C. parapsilosis	19 (24.1)	8 (26.7)	0.80
C. glabrata	12 (15.2)	3 (10.0)	0.75
C. tropicalis	4 (5.1)	0	0.58
C. krusei	3 (3.8)	3 (10.0)	0.34
Other *Candida* species	3 (3.8)	0	0.56
Source, n (%)			
Central venous catheter	46 (58.2)	19 (63.3)	0.66
Intra-abdominal	14 (17.7)	4 (13.3)	0.77
Primary	8 (10.1)	5 (16.7)	0.34
Urinary tract	6 (7.6)	1 (3.3)	0.67
Other sources[b]	5 (6.3)	1 (3.3)	1
Initial antifungal therapy, n(%)			
Fluconazole	53 (67.1)	21 (70.0)	0.30
Echinocandins	23 (25.3)	6 (20.0)	0.34
Liposomal amphotericin B	3 (3.8)	3 (10.0)	0.28
Early adequate antifungal therapy, n(%)	71 (89.9)	27 (90.0)	1
Early adequate source control of infection, n(%)	61 (95.3)	17 (73.9)	**0.009**
Persistent candidemia, n(%)	16 (20.5)	7 (30.4)	0.39
ICU admission due to candidemia, n(%)	3 (3.8)	7 (23.3)	**0.004**
Intervention period, n(%)	41 (51.9)	12 (40.0)	0.29
All bundle elements successfully completed, n(%)	55 (69.6)	15 (50.0)	0.07

[a] Other Candida species include: 1 *C. lusitaniae*, 1 *C. dublinensis* and 1 *C. incospicua*

[b] Other sources include: 1 chorioamnionitis, 2 peripheral catather. 2 infective endocarditis, 1 infection from prosthesis

ICU, intensive care units

*P values < 0.05 are shown in bold

TABLE 13. Cox regression analyses of Variables Associated With 14- Mortality Among Patients With *Candida* BSI

	14-DAY MORTALITY			**30-DAY MORTALITY**		
	HR	95% Confidence interval	*p* Value*	HR	95% Confidence interval	*p* Value*
Septic Shock due to candidemia	**11.6**	**1.18-113.97**	**0.04**	2.04	0.62-6.73	0.24
Primary candidemia	**4.83**	**1.40-16.69**	**0.01**	2.82	0.94-8.44	0.06
ICU admission due to candidemia	3.92	0.90-16.21	0.06	**4.60**	**1.62-13.02**	**0.004**
Age ≥ 65 years	2.95	0.73-11.85	0.12	**2.6**	**1.10-6.33**	**0.03**
Male	1.47	0.40-5.38	0.55	1.57	0.63-3.92	0.33
Pitt score	0.62	0.32-1.17	0.14	1.00	0.80-1.24	0.98
Surgical ward	0.28	0.06-1.30	0.10	**0.14**	**0.04-0.51**	**0.003**
Intervention period	**0.08**	**0.01-0.45**	**0.004**	**0.40**	**0.18-0.89**	**0.02**

*P values < 0.05 are shown in bold

DISCUSSION

In the third study of the present thesis we show that the combination of a comprehensive candidemia bundle with an antifungal stewardship program involvement significantly improve adherence to guidelines recommendations and overall management of patients with candidemia. This novel approach was effective and resulted in a reduction in 14-day and 30-day all-cause mortality in the post-intervention group.

To our knowledge, this study is the largest and most inclusive study evaluating the clinical impact of a check list care bundle for management of patients with candidemia. Even though, many published studies have described individual aspects of the bundle being associated with better prognosis (i.e. early adequate antifungal administration or adequate source control) [13, 36, 63, 118]., only three recent studies have addressed the impact of a bundle approach on the management of patients with candidemia [122-124]. Antworth et al [122] conducted a single-center study of 78 patients with candidemia and demonstrated improved compliance with all candidemia care bundle elements, but not significant differences in clinical outcome.

Takesue and collegues [123] performed a nationwide study of 608 patients with candidemia to investigate compliance with their bundle and its impact on mortality. Unfortunately, study participation by infection control doctors was entirely voluntary and the candidemia bundle was not systematically implemented to all candidemic patients. Reflecting this weakness, compliance with all bundle elements was particularly poor (6.9%) and correlation between bundle compliance and either clinical success or mortality was not observed.

Finally, Gouliorius et al. implemented a similar study design to ours, but lacked the sample size to detect significant differences in mortality (12/44 [27%] versus 3/33 [9%], p=0.08) [124].

In our study, the overall compliance to quality indicators was significantly improved in the intervention group, mainly driven by improvements in the administration of early adequate antifungal therapy, early source control of

infection and adequate length of antifungal therapy. Noteworthy, the improvements were demonstrated in a clinical setting with a long experience in antifungal stewardship program [61, 65, 102], in which the comparatively pre-intervention adherence to recommendations was particularly high (80.4-89.3%). These results highlight the importance of candidemia bundle implementation even in institutions with high baseline adherence and not only in hospitals with low baseline rates of bundle adherence, where the impact of the intervention could be even better than ours.

Regarding this aspect, the current study is the first demonstrating how the systematic implementation of a candidemia bundle can be associated with decreased all cause of mortality. Although previous studies showed that infectious disease consultation was associated with better management and patient outcome [102, 119, 120], we surprisingly found that 89.2% of the patients in the pre-intervention period were visited by an infectious disease specialists. We believe that the use of a structured “checking list” used for making recommendations was crucial for reminding the infectious disease specialists of all the key aspects to consider for the management of patients with candidemia.

It is important to mention that other reasons could explain the better evolution of patients treated according to our candidemia bundle. First, our bundle was unique in that we not only looked at the intervention of implementing a candidemia protocol, but also looked at multiple aspects in the process of care, including management of other concomitant infections, medication toxicity and drug-drug interactions. All these items could have

contributed to a better use of antifungal therapy, especially in a population with multiple comorbidities such as ours. Secondly, in our bundle we included as mayor recommendation an “adequate source control of the infection” rather than the “simple” CVC withdrawal included in all earlier studies [122-124]. As previously reported, the benefit of early CVC withdrawal might be disputable when the source of candidemia is not the catheter [62, 125]. Considering that 30-40% of candidemic patients had an origin other than CVC, the more restrictive definition of adequate source control used by Antworth, Takesue and Goulorius [122-124] may explain why their reports were unable to find a significant association between bundle interventions and mortality.

Finally, in contrast to previous studies, we did not include “de-escalation to fluconazole” [124] or “step down therapy” [123] as care element of our bundle. Despite these components may play a significant role in term of length of hospitalization and costs, its role in term of improvement of patient outcome could be limited. In our opinion, future studies should be performed to compare candidemia bundles in order to identify one that is easier to perform and associated with a better outcome.

This study has some limitations that should be assessed. First, it is a quasi- experimental pre-post study design that lacks of randomization. Thus, we may not have taken into account changes in standard of care for patients with candidemia during the study periods. Second, this is a single center study and our findings may not be applicable to other settings. Third, our outcome

measure was all-cause mortality, and we did not report any data on *Candida* attributable mortality or mycological response.

In conclusions, the introduction of a comprehensive candidemia bundle with antifungal stewardship program involvement significantly improved adherence to quality indicators, overall management and clinical evolution of patients with candidemia. Our study encourage the systematic use of care bundles for the management of candidemia.

VII. CUARTO OBJETIVO:

Monitorización terapéutica de fármacos antifúngicos: ¿otra herramienta para mejorar el desenlace clínico del paciente?

Therapeutic drug monitoring of antifungal drugs: another tool to improve patient outcome?

INTRODUCTION

Invasive fungal infections (IFIs) remain a major clinical concern because of their increasing incidence, high morbidity and mortality rates [114, 126]. Adequate treatment requires proper drug selection and proper dosing of antifungal drugs [102, 127, 128].

Triazoles or echinocandins are the most commonly used drugs for preventing or treating IFI [129] and failure to achieve adequate serum concentrations has been advocated as a possible cause of poor outcomes [129, 130], emergence of resistance [131],and toxicity [132, 133]. Nevertheless, systematic therapeutic drug monitoring (TDM) of antifungals is not considered routinely necessary [129], mainly due to the belief that adequate antifungal serum concentration is usually achieved by prescribing fixed doses according to international guidelines [73]. Unfortunately, evidences supporting this assumption are scarce and mainly related to specific patients groups [134-137] or antifungals class [138-148].

The aim of this study was to determine whether doses of antifungal drugs accurately predict serum concentration in a non-selected group of hospitalized patients. We also tried to evaluate the impact of inadequate dosage or inadequate antifungal serum concentrations on clinical outcome.

MATERIALS AND METHODS

This was a prospective observational cross-sectional study performed from March 2015 to June 2015 in our 1,550-bed tertiary care hospital that

serves a population of approximately 715,000 inhabitants. It is a referral center for solid organ transplantation, heart surgery, stem cell transplantation and HIV/AIDS care.

During the study period, the list of the patients who were starting systemic antifungal treatment was daily received from the Pharmacy department. The choice of the antifungal agent as well as dosing was made by the attending physicians who were unaware about the study design. No feedback was maintained with them until the end of the study.

All consecutive non-selected adult patients who received a systemic triazole or an echinocandin for prophylaxis or treatment (either empirical or targeted) of IFI were included in the study if they gave their written informed consent and a blood sample for TDM was drawn. According to study protocol each patient had one blood sample drawn at least 3 days post-initiation of treatment. Trough levels were obtained within 30 minutes before dosing. Adequacy of antifungal dosing and serum concentration as well as clinical outcome was evaluated at discharge of the patient.

The study was approved by the Local Ethical Committee “Comité Ético de Investigación Clínica, Hospital General Universitario Gregorio Marañón” (number 2015-066).

Data collection and definition

The following data were prospectively collected using a standardized case report form: sex; age; weight and height; Charlson comorbidity index; renal

and hepatic function (serum creatinine and creatinine clearance); presence of extracorporeal devices such as continuous renal replacement therapy (CRRT) or extracorporeal membrane oxygenation (ECMO); risk factors for IFI (i.e. presence of central venous catheter, parenteral nutrition, corticosteroid therapy, recent surgery); indication for antifungal treatment; type of antifungal drug; dosage; microbiological findings and clinical evolution of the patients.

IFI related mortality was defined as the death that could be attributed to IFI as either the immediate or underlying cause.

Clinical outcome

Clinical outcome was considered favorable when the following criteria were fulfilled: completion of treatment course without broadening antifungal spectrum or addition of another antifungal drug; no evidence of breakthrough IFI; and/or no evidence of IFI related mortality.

Antifungal drug administration and sample collection

The adequacy of antifungal dosage was defined according to current IDSA guidelines [15] and the classification of antifungal dosage regimens in adequate or inadequate is shown in the **Supplementary material.** Dose adjustment for hepatic and/or renal dysfunction and drug-drug interactions were also taken into consideration, when necessary.

Serum antifungal concentrations were determined with high-performance liquid chromatography. Samples were processed as previously described by

Arendrup et al. for anidulafungin and caspofungin [149], Gordien et al. for triazoles [150], and Martens-Lobenhoffer et al. for micafungin [151]. According to pharmacokinetics data, the following through serum concentration were considered within therapeutic range: fluconazole >11 µg/ml [137], echinocandins >1 µg/ml [152], voriconazole 1-5.5 µg/ml [153] and posaconazole >0.7 µg/ml [129, 154]. Precision and accuracy were assessed by performing replicate analysis of quality control samples against calibration standards.

STATISTICAL ANALYSIS

The total number (and percentage) of cases with trough concentrations out of therapeutic target for each antifungal drug was assessed. Continuous variables are presented as mean value (± SD) or median values (range) for normally or non-normally distributed data. Categorical variables are expressed as frequency and percentage.

To identify independent predictors of though serum antifungal concentration we performed univariate and multivariate linear regression analyses, including in the multivariate stepwise backward analysis, all the variables significant at $p \leq 0.200$ in the univariate analysis.

A multivariate logistic regression model was used to assess the independent effect of either the adequate serum antifungal concentration or the adequate antifungal dose according to guidelines on the outcome of patients with IFI. A forward stepwise approach was followed, including as candidate variables all those that were significant in the univariate analysis. The results

are presented as adjusted odds ratios with 95% confidence intervals. All statistical procedures were performed using SPSS version 15.0 (SPSS Inc., Chicago, IL, USA).

RESULTS

Overall, 84 patients were included in the study. Most of them were male (n= 55, 65.4%) and the mean age (±SD) was 59.6 years (± 14.1). Hospital admission wards, main underlying diseases, associated risk factors and indications for antifungal therapy are summarized in **Table 14**.

Antifungal drugs were prescribed as prophylaxis in 34 patients (40.4%), targeted therapy in 26 (31.0%) and empirical therapy in 24 (28.6%). *Candida* blood stream infection (n=11), followed by pulmonary aspergillosis (n=8) and intra-abdominal infection (n=7) were the most common proven IFI.

Table 14. Demographic and Clinical Characteristics of Patients

CHARACTERISTICS	N=84
Age, years, mean ± SD	59.6 ± 14.1
Male sex, %	55 (65.4)
Charlson comorbidity index	4.0 ± 2.9
Hospital department, %	
Onco-hematology	28 (33.4)
Intensive care units	22 (26.2)
Internal medicine	20 (23.7)
Surgical department	14 (16.7)
IFI risk factors	
Immunosuppression, %	46 (54.8)
Leukemia/Lymphoma	22 (26.2)
Solid organ cancer with chemotherapy / radiotherapy	15 (17.9)
Hematopoietic stem cell transplant	11 (13.1)
HIV infection	11 (13.1)
Other, %	
Central venous catheter	62 (73.8)
Surgery in the last 3 months	37 (44.0)
Corticosteroids	33 (39.3)
Total parenteral nutrition	28 (33.3)
Continuous renal replacement therapy	3 (6.4)
Other underlying disease,%	
Renal disease	17 (20.2)
Liver disease	10 (11.9)
Indication for Antifungal therapy,%	
Prophylaxis	34 (40.4)
Empirical therapy	24 (28.6)
Targeted therapy	26 (31.0)
Adequate AF dosage according to guidelines,%	76 (90.5)

Adequate AF serum concentration,%	54 (64.3)
Final diagnosis (patients with targeted indication, n=26),%	
Candida BSI	11/26 (42.3)
Invasive aspergillosis	8/26 (30.7)
Intra-abdominal candidiasis	7/26 (26.9)

AF antifungals; **BSI** bloodstream infection

Correlation of appropriate trough serum concentrations and antifungal dosage

Among the 84 patients, the most frequently used antifungal agent was fluconazole 400 mg (16/84, 19.0%) followed by voriconazole, (15/84, 17.8%), micafungin 100 mg (14/84, 16.7%), micafungin 50 mg (14/84, 16.7%), anidulafungin (8/84, 9.5%), posaconazole (7/84, 8.4%), fluconazole 200 mg (6/84, 7.1%) and caspofungin (3/84, 3.6%). According to current guidelines, antifungal dosages were classified as appropriate in 76 out of 84 patients (90.5%).

As shown in **Table 15** we observed a large inter-individual variability in trough serum concentration with all drugs: echinocandins ranged from 0 to 7.2 µg/ml, fluconazole from 1.9 to 47.7 µg/ml, voriconazole from 1.1 to 11.0 µg/ml, and posaconazole from 0.2 to 2.2 µg/ml. Therefore, an adequate exposure according to serum concentration was reached in only 54/84 cases (64.3%). The proportion of samples with on-target serum levels were as follows: anidulafungin (8/8; 100%), voriconazole (13/15, 86.7%), fluconazole 400 mg (12/17, 70.5%), caspofungin (2/3, 66.7%), micafungin 100 mg (9/14, 64.2%),

posaconazole (4/7, 57.1%), micafungin 50 mg (5/14, 35.7%) or fluconazole 200 mg (1/6, 16.7%)

When we specifically analyzed patients receiving an adequate dose of antifungal according to current guidelines (76/84, 90.5%), we found that again only, 67.1% of them (51/76) attained an adequate serum concentration. Of the remaining 8/84 (9.5%) patients (all receiving lower than recommended antifungals dosage), 3 of them attained on-target serum antifungal level and 5 did not

Table 15. Clinical data and adequacy of antifungal dose according to serum concentration and current guidelines.

	Overall 84	**Fluconazole 200 mg (n=6)**	**Fluconazole 400 mg (n=17)**	**Voriconazole (n=15)**	**Posaconazole (n=7)**	**Micafungin 50 mg (n=14)**	**Micafungin 100 mg (n=14)**	**Anidulafungin 100 mg (n=8)**	**Caspofungin 50 mg (n=3)**
Factors potentially influencing antifungal serum concentration									
Male Sex	55 (65.4)	3 (50.0)	9 (52.9)	11 (73.3)	3 (42.9)	9 (64.3)	12 (85.7)	6 (75.0)	2 (66.7)
Weight (Kg), median (range)	66 (40-117)	59 (40-68)	62 (40-88)	64 (40- 96)	69 (56-78)	84.5 (48-87)	67 (44-95)	75 (71-79)	70 (68- 117)
Body surface area	1.70 ± 0.34	1.60 ± 0.15	1.59 ± 0.46	1.63 ± 0.50	1.74 ± 0.13	1.73 ± 0.20	1.78 ± 0.25	1.86 ± 0.11	2.26 ± 0.23
CL_{CR} (ml/min), median (IQR)	89.0 (48.3 -116.4)	87.2 (64.4- 140.5)	84.1 (44.2-84.1)	82.1 (67.8-115.2)	86.5 (49.6-105.1)	109.0 (94.5-126.7)	47.2 (24.5-106.8)	72.9 (35.1-183.6)	103 .3 (NE)
Serum albumin (mg/dl), median (IQR)	3.0 (2.5-3.5)	2.3 (2.0 -2.5)	2.6 (2.0-3.0)	3.6 (3.3-4.0)	3.3 (2.8-3.6)	3.3 (3.0-3.9)	2.7 (2.4-3.2)	2.4 (2-2.9)	2.8 (NE)
Creatinine (mg/dl), median (IQR)	0.8 (0.6-1.1)	0.6 (0.4-0.8)	0.7 (0.5- 0.9)	0.9 (0.6- 0.94)	0.8 (0.7-1.4)	0.7 (0.5-0.9)	1.3 (0.8-2.5)	0.8 (0.6-2.5)	0.7 (NE)
Intensive care unit stay	22 (26.2)	1 (16.7)	3 (17.6)	1 (6.7)	1 (14.3)	0 (0)	7 (50.0)	6 (75.0)	3 (100)
Adequancy of serum antifungal concentration									
According to serum concentration	54 (64.3)	1 (16.7)	12 (70.6)	13 (86.7)	4 (57.1)	5 (35.7)	9 (64.3)	8 (100)	2 (66.7)
According to current guidelines	76 (90.5)	2 (33.3)	16 (94.1)	13 (86.7)	7 (100)	14 (100)	13 (92.9)	8 (100)	3 (100)
Serum AF concentration (µg/ml) , median (range)	NA	7.0 (1.9-15.3)	15.09 (4.3-47.0)	3.2 (1.1-11.0)	0.8 (0.2-2.2)	0.83 (0- 2.27)	1.2 (0.6- 7.13)	2.3 (1.1- 2.8)	3.25 (0-4.7)

AF antifungals, **IQR** interquartile range; **NE** not evaluable

Variables associated with through level of antifungals

Variables associated with trough level of antifungals are shown in **Table 16.** Univariate analysis showed that age, male sex, weight, body surface and daily dose of antifungals were all variables associated with though concentration, either by augmenting drug exposure (older age, daily dose), or lowering it (male sex, weight and body surface area). Multivariate analysis confirmed age and daily dose as factors associated with an increased drug exposure, whereas the body surface area correlated with a decrease in serum antifungal concentrations. We were not able to demonstrate a significant correlation between renal function and though serum antifungal concentration.

Interestingly, when we separately analyzed each antifungal class administered in our study, no associations were found between any variables and though serum concentration for voriconazole, posaconazole, caspofungin and anidulafungin. However, serum albumin level was correlated with fluconazole serum concentration (by increasing it), whereas male sex, daily dose, body surface area and serum albumin were the variables showing significant correlation with the trough micafungin concentration **(Table 17).**

Table 16. Univariate and multivariate analysis of variables associated with through level of all antifungals (n=84)

	Univariate analysis		**Multivariate analysis**	
	Unstandardized β- coefficient (95% CI)	***p***	**Unstandardized β- coefficient (95% CI)**	***p***
Age (years)	**0.20 (0.07, 0.33)**	**<0.01**	**0.16 (0.06-027)**	**0.003**
Male sex	**-4.29 (-0.32, -8.26)**	**0.03**	-	-
Weight (kg)	**-0.11 (-0.21,-0.01)**	**0.03**	-	-
Body Surface area	**-6.31 (-11.89, -7.32)**	**0.03**	**-10.25 (-17.42, -3.09)**	**0.006**
CL_{CR} (mL/min)	-0.02 (-0.04, 0.01)	0.19	-	-
Daily dose (mg/kg)	**0.04 (0.02 – 0.04)**	**<0.01**	**0.03 (0.02- 0.04)**	**0.001**
Intensive care Unit stay	-2.41 (-6.79; 1.96)	0.27	-	-
Serum albumin, mg/dl	-1.62 (-4.4; 1.17)	0.25	-	-
Charlson comorbidity index	0.04 (0.31, 1.10)	0.26	-	-

Table 17. Multivariate analysis of variables associated with through level of all Fluconazole (n=23), and micafungin (n=28).

	Multivariate analysis Fluconazole		**Multivariate analysis Micafungin**	
	Unstandardized β- coefficient (95% CI)	***p***	**Unstandardized β- coefficient (95% CI)**	***p***
Age (years)	-	-	**0.16 (0.06-027)**	**0.003**
Male sex	-	-	**-1.48 (0.57, 2.44)**	**<0.01**
Weight (kg)	-	-	**-**	**-**
Body Surface area	-	-	-	-
CL_{CR} (mL/min)	-	-	-	-
Daily dose (mg/kg)	-	-	**0.03 (0.01, 0.47)**	**<0.01**
Intensive care Unit stay	-	-	-	-
Serum albumin, mg/dl	**-**	**-**	**0.72 (0.33, 1.48)**	**0.04**

Clinical evolution

Overall, a favorable clinical outcome was observed in 77/84 patients (91.6%), following a median duration of therapy of 15 days. Factors associated with a poor outcome in the univariate analysis were high Charlson comorbidity index (p=0.016), previous treatment with corticosteroids therapy (p=0.01) and total parenteral nutrition (p=0.04). Adequate serum antifungal concentration tended to be associated with a favorable outcome (p=0.09) **(Table 18)**, whereas no correlation between clinical outcome and adequate antifungal dosage was observed in univariate analysis.

Multivariate analysis **(Table 18)**, showed that attaining on-target serum antifungal level was significantly associated with a favorable clinical outcome (OR=0.02; 95% CI 0.01–0.64; P = 0.03). Conversely, the administration of an adequate antifungal dosage according to current guidelines was not associated with a favourable clinical outcome (OR=1.12; 95% CI 0.03-40.6, p=0.93).

Table 18. Univariate and multivariate analysis for risk factors for Poor clinical outcome

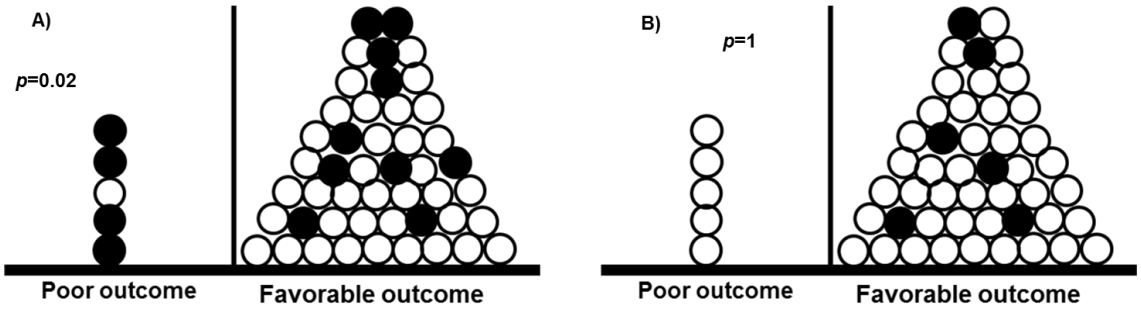
CHARACTERISTICS	Good clinical outcome (n=77,%)	Poor clinical outcome (n=7,%)	*p*	Multivariate analysis OR (95% CI)	p
Age, years, mean ± SD	59.8 ± 14.1	57.9 ± 15.7	0.73	-	-
Male sex, %	50 (64.9)	5 (71.4)	1	-	-
Charlson comorbidity index	**3.7 ± 2.5**	**6.3 ± 3.4**	**0.016**	0.6 (0.3-1.1)	0.09
IFI risk factors					
Immunosuppression, %	42 (54.5)	4 (57.1)	1	-	-
Leukemia/Lymphoma	22 (28.6)	0	0.18	-	-
Solid organ cancer with chemotherapy /	13 (16.9)	2 (28.6)	0.60	-	-
radiotherapy	10 (13.0)	1 (14.3)	1	-	-
Hematopoietic stem cell transplant	10 (13.0)	1 (14.3)	1	-	-
HIV infection					-
Other, %	56 (72.7)	6 (85.7)	0.67	-	-
Central venous catheter	33 (42.9)	4 (57.1)	0.69	-	-
Surgery in the last 3 months	**27 (35.1)**	**6 (85.7)**	**0.01**	14.6 (0.98- 220.6)	0.06

Corticosteroids	**23 (29.9)**	**5 (71.4)**	**0.04**	2.74 (0.28- 26.39)	0.38
Total parenteral nutrition	3 (7.1)	0	1	-	-
Continuous renal replacement therapy					
Indication for Antifungal therapy,%					
Prophylaxis	32 (41.6)	2 (28.6)	0.60	**-**	**-**
Empirical therapy	23 (29.9)	1 (14.3)	0.7	**-**	**-**
Targeted therapy	22 (28.6)	4 (57.1)	1	**-**	**-**
Adequate AF dosage according to guidelines,%	69 (89.6)	7 (100)	1	1.12 (0.03- 40.6)	0.93
Adequate AF serum concentration,%	52 (67.5)	2 (28.6)	0.09	**0.02 (0.01- 0.64)**	**0.03**
Final diagnosis (patients with targeted indication),%					-
***Candida* BSI**	9 (11.7)	2 (28.6)	0.22	-	-
Invasive aspergillosis	6 (7.8)	2 (28.6)	0.13	-	-
Intra-abdominal candidiasis	7 (9.1)	0	1	-	-
Creatinine clearance	97.5 ± 73.2	82.9 ± 56.7	0.60	-	-
Length of antifungal therapy	33.2 ± 32.2	18.2 ± 20.1	0.31	-	-

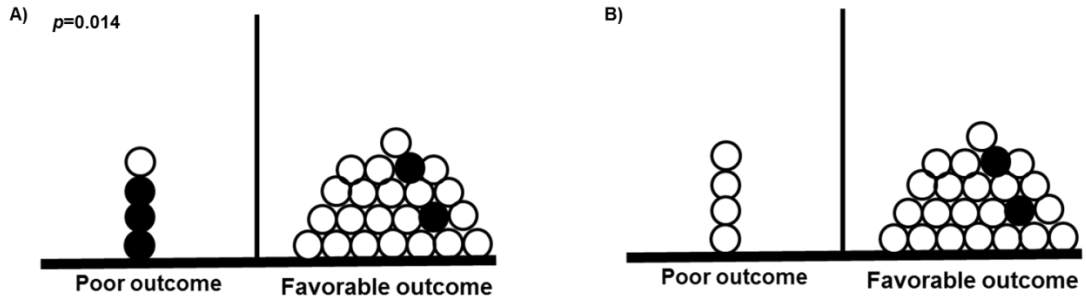
When we performed an additional analysis including only patients with empirical or targeted therapy, both univariate and multivariate analysis showed a strong correlation between favourable clinical outcome and serum antifungal drug concentration (**Figure 3**).

FIGURE 3 Clinical outcome of patients according to adequate serum concentration (A) or current guidelines (B)

1A) Patients with empirical and targeted therapy (n=50)



1B) Patients with targeted therapy were included (n=26)



Every patient is represented with a dot.

Black dots for patients who did not achieve adequate AF exposure

White dots for those who did it

DISCUSSION

The findings of the fourth research suggest that, in a non-selected group of hospitalized patients receiving triazoles or echinocandins, there is a poor correlation between guidelines-based antifungal dosage and adequate serum drug concentrations, with a large proportion of patients being outside therapeutic target. Moreover, an adequate antifungal serum concentration seems to better predict the clinical outcome of the patients, thus advising performance of TDM of all antifungals at least in old patients and those with hypoalbuminemia.

The mortality of patients with IFI is reported to be between 20% and 50% [129]. Risk factors for poor prognosis include the specific type of invasive fungal infection [155], and patient or treatment-related factors (older age, neutropenia, malignancies, liver disease, delay in appropriate treatment) [155, 156]. Until recently, the effect of therapeutic drug monitoring was not perceived as a need for improving prognosis in patients receiving adequate antifungal doses [15].

Both the IDSA [15] and the British Society of Medical Mycology guidelines [154] recommend systematic TDM in patients receiving posaconazole or voriconazole due to their pharmacokinetics variability [157] and potential relationship between serum drugs concentration and therapeutic efficacy [145, 147] or toxicity [158]. On the other hand, the same guidelines do not support systematic TDM of fluconazole and echinocandins [154] because of the linear and predictable

pharmacokinetics profile [152, 159] as demonstrated by studies performed in vitro and in healthy volunteers.

Nevertheless, when the pharmacokinetics of fluconazole, anidulafungin and caspofungin were prospectively addressed in critically ill patients receiving fixed doses of antifungals, a considerable interindividual variability was observed, with a large proportion of patients (up to 33%) not attaining the optimal pharmacokinetics/pharmacodynamics target [135].

In the same sense, our data also demonstrates a high variability of antifungals exposure. Accordingly, it is not possible to predict a priori the antifungal concentrations achieved in a particular patient, suggesting that it may be necessary to ascertain the drug concentrations reached. Regarding this aspect, during the study period, 90.5% of patients receiving antifungals at our center were treated appropriately, according to current guidelines. However, only 67.1% of them had an adequate antifungal serum level, thus supporting the role of systematic TDM for optimizing antifungal treatment.

The high variability of the levels and the low correlation between the dosage administered and the serum concentration may be attributed to different aspects including inconsistent absorption [160, 161], body weight [162] and composition [163], genetic polymorphism and metabolism [164], elimination [153] or interaction between different drugs [161 165]. This may be especially relevant for specific type of patients such as critically ill or hematological patients [134, 135, 137], which represents the most important population of in our cohort. An in-depth

analysis of factors affecting the trough serum concentration of antifungals in our population showed that most of the inter-patient variability could be explained by demographics characteristics (age and body surface area). Although we found a significant positive correlation of antifungal dosage in all the population, this fact was due to the patients receiving micafungin who were the only group with different doses. Our analysis also showed a positive correlation between serum albumin concentrations and fluconazole and echinocandins exposures. Nguyen et al. [138-148], reported that low serum albumin concentrations in the surgical ICU patients were correlated with low caspofungin exposures. We believe that patients with hypoalbuminemia should be considered as a "high risk group" of low antifungal serum exposure that could especially benefit of systematic monitorization of antifungals concentration.

Many authors in the last two decades **(supplementary material 2)** have tried to assess a relationship between serum antifungal exposure and clinical outcome [137-148, 166-170]. Although, the benefit of voriconazole TDM was established by different studies [138-148], including a randomized controlled clinical trial [166]. However, very few studies have analyzed the impact of fluconazole or posaconazole [136, 137, 171] TDM on clinic outcome of patients with IFI. Moreover, to the best of our knowledge, studies evaluating this aspect in patients treated with echinocandins have not been performed yet. As for fluconazole, a retrospective study in the Netherlands including 99 critically ill children (46 with a proven invasive fungal infection), found a positive association between

fluconazole trough concentration and a shorter time of culture conversion. In another study, Manosuthi et al. [136] examined 64 HIV infected patients with cryptococcal meningitis treated with a combination therapy of fluconazole at different dosage plus amphotericin B. They found that patients with a high serum and CFS fluconazole concentration exhibited a higher rate of survival. Similarly, in a study performed in 17 patients receiving posaconazole (6 with possible /proven IFI) the authors found that a serum concentration ≥0.5 μg/ml was associated with a successful outcome [171].

The results of our study suggest that adequate serum antifungal concentration may be an additional tool for improving the clinical outcome of patients with suspected or confirmed IFI. We were unable to demonstrate a clinical correlation between with an adequate antifungal dosage and the clinical outcome. Interestingly, the relationship between favourable clinical outcome and adequate antifungal serum concentrations seemed to be stronger when only patients receiving antifungal empirical or targeted therapy were analysed. A possible explanation could be the fact that the wide use of active antifungal prophylaxis has significantly reduced to less than 5% the rate of breakthrough IFI [172], that was the only factor associated with poor outcome in the prophylaxis sub-group.

Our study has some limitations. First, in this proof of concept study we evaluated the determination of only one antifungal concentration per patient, without estimating pharmacodynamics parameters. Second, although we used

previously proposed cut-offs for fluconazole and echinocandins TDM [137, 152], we are aware that adequate through serum concentrations for such drugs have not been yet established. Third, we did not record relevant drug-drug interactions that could explain, at least in part, the high inter-variability observed. However, this factor could have been minimized by the existence of an alert system from the Pharmacy Department that notifies immediately every possible drug-drug interaction. Lastly, the universal applicability of systematic TDM maybe limited by the availability of laboratories.

In summary, we show that with standard antifungal dosage, a considerable proportion of patients have low drug concentrations, which is associated with poor clinical outcome. If future studies confirm these data, antifungal drug monitoring should be performed routinely in hospitalized patients and doses would have to be scheduled according to the levels reached.

VIII. QUINTO OBJETIVO:

Comparación de la seguridad y eficacia de micafungina versus otras candinas y azoles en pacientes con enfermedad hepática pre-existente con Child Pugh B o C

Safety and tolerance profile of micafungin in patients with pre-existing Child-Pugh B or C liver disease

INTRODUCTION

Patients with end-stage liver disease (ESLD) are at high risk of acquiring invasive fungal infections (IFI) because of alterations in gut microbiota, gut permeability, and immune dysfunction[173, 174]. The frequency of IFI in ESLD patients, ranges from 1% to 10% [175, 176], and development of IFI has a profound effect on the outcome of ESLD [177, 178].

Echinocandins are among the better tolerated antifungals in patients with ESLD [179-186]. Nevertheless, micafungin is the only echinocandin not approved in patients with ESLD because of an EMA warning on the potential development of liver tumors, as shown in preclinical studies in rats treated with high micafungin doses for either 3 or 6 months.

In humans, data on hepatotoxicity in patients treated with micafungin are scarce [187-192] and, to our knowledge, only 3 studies have partially evaluated this issue among ESLD patients [184-186]. However, data on the incidence of long-term liver injury (LTLI) and the relative magnitude of this risk compared with other antifungals have not been reported.

We performed a large, multicenter, case-control study in order to define, in routine clinical practice, the association between exposure to micafungin, other echinocandins, or azoles and the development of short-term liver injury (STLI) or LTLI in a population of patients with pre-existing Child-Pugh B or C ESLD.

MATERIALS AND METHODS

Study design and setting

We performed a retrospective, multicenter, case-control study in 6 large tertiary-care hospitals in Spain and Italy. The study cohort consisted of adult patients with chronic Child-Pugh B or C ESLD who received ≥ 72 hours of therapy with micafungin, other echinocandins or azoles from May 2009 to May 2015.

In order to avoid bias, cases were identified in each institution based on the diagnosis and procedure codes of the *International Classification of Disease, Ninth Revision, Clinical Modification (ICD-9-CM).* Patients with a primary and secondary diagnosis of chronic liver disease (ICD-9-CM codes used for that purpose are listed in **Supplementary 2**) were identified. Data were cross-checked with pharmacy databases consisting of all patients who received an echinocandin or an azole during the study period. Information on etiology, severity of liver disease, and antifungal exposure were then re-checked using the clinical charts.

INCLUSION CRITERIA

To be considered cases, patients had to meet all of the following criteria: 1) ESLD classified as Child-Pugh B or C on the first day antifungal therapy was started; 2) micafungin at 100 mg daily for at least 72 hours; 3) prior and subsequent measurement of liver function.

For each case, we selected 2 matched controls: 1 receiving another class of echinocandins (caspofungin loading dose of 70/50 mg followed by 50/35 mg once daily or anidulafungin loading dose of 200 mg then 100 mg once daily) and 1 receiving an azole (fluconazole 400 mg once daily or voriconazole loading dose of 6 mg/kg then 4 mg/kg twice daily). To be considered matched controls, patients had to be Child-Pugh B or C ESLD and fulfill the following conditions: 1) same sex; 2) antifungal therapy at about the same time as the case; 3) antifungal therapy with the same indication as the case (empirical or targeted treatment); 4) survival for as long as the case after administration of antifungal drugs.

Patients who underwent liver transplantation were eligible for the study only if the graft was affected by a chronic disease (i.e., patient with recurrence of HCV cirrhosis after liver transplant). For both cases and controls, the index date was defined as the date of the first administration of the study drug.

Follow-up and outcomes

Outcomes were assessed during a follow-up period that began on the index date and ended on the date of death or the last clinical visit until the end of June 2016. The primary outcome of the study was the incidence of short- or long-term toxicity in patients with Child-Pugh B or C ESLD. STLI was defined as an increase during antifungal treatment in transaminase levels to >3 times the upper limit of normal for patients who started antifungal therapy with normal liver function. If patients started antifungal treatment with abnormal baseline transaminase levels (i.e., >50% greater than the upper limit of normal), STLI was defined as a doubling of the baseline transaminase level. LTLI was defined as the development of any type of liver tumor during the follow-up period.

Secondary outcomes were cumulative incidence of patients stopping treatment owing to abnormal liver function, cumulative incidence of patients needing transplantation owing to hepatotoxicity, re-admission for any cause in the following year, and number of episodes of ascitic decompensation or gastrointestinal bleeding during the following year.

Clinical Data

Data collected included demographic data, etiology of liver disease, underlying disease, and clinical course. When available laboratory data were collected at days –7, –3, 0, +1, +3, +5, +7, +14, +28, +45, +60, +120, and +180 and included the international normalized ratio (INR), aspartate aminotransferase

(AST), alanine aminotransferase (ALT), alkaline phosphatase (ALP), total bilirubin, gamma glutamyl transferase (GGT), alkaline phosphatase (ALP), creatinine, and albumin. Detailed data were also collected on concomitant drugs, type of invasive fungal infection, and pathogens.

Ethics

The study was approved by our institutional review board, which waived the need for informed consent owing to the retrospective design of the study.

Statistical analysis

Patients who received micafungin were compared with those who received other echinocandins or azoles. To detect significant differences between groups, we used the chi-square test or Fisher exact test for categorical variables and a 2-tailed *t* test or Mann-Whitney test for continuous variables, when appropriate. Values are expressed as mean ± standard deviation (continuous variables) or as percentages of the group from which they were derived (categorical variable).

A multivariable logistic regression analysis was performed to assess risk factors for STLI. Variables associated with the development of STLI in the univariate analysis (P value ≤0.3) were selected for possible inclusion. Statistical significance was set at $P < 0.05$. The results were analyzed using SPSS, version 17.0 (SPSS Inc., Chicago, IL, USA).

RESULTS

Between May 2009 and May 2015 (6 years), 2,335 patients with a diagnosis of chronic liver disease were admitted to the 6 study centers (Hospital General Universitario Gregorio Marañón, 537 patients; Nuovo Santa Chiara University Hospital, 520; Santa Maria Misericordia Hospital, 450; Hospital Universitario Ramón y Cajal, 400; Hospital Puerta de Hierro, 260; and Hospital del Mar, 168). Of these, only 20 patients who fulfilled the criteria of Child-Pugh B or C liver disease received micafungin for ≥72 hours. Overall, patients receiving micafungin represented 0.85% of all those with a diagnosis of chronic liver disease.

Comparison of ESLD patients receiving micafungin with those treated with other echinocandins or azoles

The demographics and baseline characteristics of the 3 groups (20 patients each) selected for the case-control study are summarized in **Table 19**. Univariate analysis revealed no significant differences between cases and controls regarding etiology of liver disease, other comorbidities, previous antibiotic therapy, and rate of cirrhosis-related complications. However, when compared with patients who received azoles, those with micafungin and other echinocandins had a higher MELD score and a higher Child-Pugh score. No differences were detected between patients who received micafungin and other

echinocandins although those with micafungin were significantly older (61.2 vs 52.8,p=0.01).

TABLE 19 Clinical characteristics of the study population

CHARACTERISTICS	Total N=60	Micafungin n=20	Other echinocandins n=20	Azoles n=20	*p*
Age, years (mean ± SD)	58.2 ± 14.5	61.2 ± 11.2	52.8 ± 9.6	60.6 ± 20.1	0.13
Male sex	45 (75.0)	15 (75.0)	15 (75.0)	15 (75.0)	1
Race					
White	59 (98.3)	20 (100)	20 (100)	19 (95.0)	1
Non-white	1 (1.7)	0	0	1 (5.0)	
Pre-existing liver disease					
HCV-associated cirrhosis	26 (43.3)	7 (35.0)	12 (60.0)	7(35.0)	0.64
HBV-associated cirrhosis	7 (11.6)	3 (15.0)	1 (5.0)	3 (15.0)	0.11
Alcohol-associated cirrhosis	18 (30.0)	7 (35.0)	5 (25.0)	6 (30.0)	0.52
Cryptogenic cirrhosis	4 (6.6)	0	1 (5.0)	3 (15.0)	0.78
Hepatocellular carcinoma	8 (13.3)	2 (10.0)	4 (20.0)	2 (20.0)	0.15
Other causes*	7 (11.6)	3 (15.0)	3 (15.0)	1 (5.0)	0.56
Baseline MELD score	17.7 ± 7.3	18.6 ± 7.6	19.6 ± 5.8	14.9 ± 7.9	0.10
Baseline Child-Pugh Class B	35 (58.3)	12 (60.0)	8 (40.0)	15 (75.0)	0.08

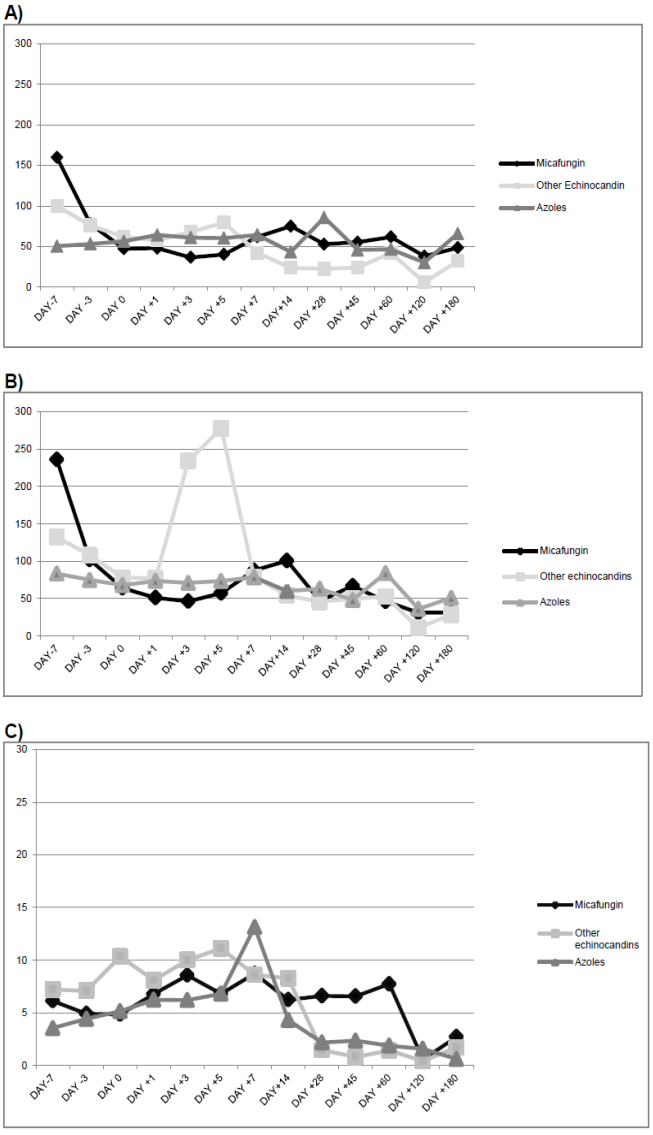
Baseline Child-Pugh Class C	25 (41.6)	8 (40.0)	12 (60.0)	5 (25.0)	0.08
Baseline Child-Pugh score	9.1 ± 1.4	9.1 ± 1.4	9.6 ± 1.2	8.6 ± 9.1	0.07
Complications within previous year					
Episode of ascites	17 (28.3)	5 (25.0)	8 (40.0)	4 (20.0)	0.34
Episodes of gastrointestinal bleeding	5 (8.3)	1 (5.0)	2 (10.0)	2 (10.0)	0.80
Antibiotic exposure	46 (76.6)	14 (70.0)	18 (90.0)	14 (70.0)	0.22
Other comorbidities					
Heart failure	6 (10.0)	3 (15.0)	2 (10.0)	1 (5.0)	0.57
Renal chronic disease	12 (20.0)	4 (20.0)	6 (30.0)	2 (10.0)	0.28
Respiratory disease	7 (11.6)	3 (15.0)	2 (10.0)	2 (10.0)	0.85
Diabetes mellitus	16 (26.6)	8 (40.0)	6 (30.0)	2 (10.0)	0.09
Cancer	12 (20.0)	6 (30.0)	3 (15.0)	3 (15.0)	0.39
HIV infection	5 (8.3)	3 (15.0)	1 (5.0)	1 (5.0)	0.41

*Hepatocellular carcinoma **AD: ascitic decompensation

Comparison between STLI and LTLI

Exposure to antifungal treatment is reported in **Table 20**. Antifungals were used as targeted therapy in most cases, and length of therapy was significantly longer among patients receiving azoles (mean duration 19.2 days) than among those treated with micafungin (12.3 days) or other echinocandins (10.9 days). On contrary, compared to azoles, septic shock was more frequent in patients who received micafungin (35% vs 0%, p=0.08) or other echinocandins (50% vs 0%, p=0.03). Six of 60 patients (10%) patients developed STLI: 2 patients with micafungin, 2 patients with other echinocandins, and 2 patients with azoles. The rate of STLI was 0.81 cases per 100 patient-days for micafungin, 0.91 cases per 100 patient-days for other echinocandins, and 0.51 cases per 100 patient-days for azoles. The increase in serum

Figure 4. Mean AST (A), mean ALT (B), and mean bilirubin (C) level accorc specific study drug.



aminotransferase was asymptomatic in all patients who experienced STLI, and antifungal discontinuation was required in 2 cases only: one patient who was receiving micafungin and another who was receiving azoles. **Figure 4** shows how laboratory values changed over time. There were no relevant differences in liver function over time between the groups. Interestingly, in all evaluable patients, transaminase levels returned to normal after withdrawal or switching of antifungal therapy. No patients developed acute liver insufficiency requiring ICU admission or liver transplantation.

Overall, in-hospital mortality was 35% for cases treated with micafungin, 45% for other echinocandins, and 25% for azoles (p=0.39). No deaths were considered related to the antifungal drugs. Causes of death were mostly related to worsening of underlying disease (9 patients), invasive fungal infection (6 patients).

Follow-up information was available for 30 patients until a median of 1.3 years after discharge. During the following year, no differences were observed between groups with respect to rate of re-hospitalization (54.1% for micafungin vs 85.7% for other echinocandins and 81.8% for azoles, p=0.23), number of episodes of ascitic decompensation (18.2% vs 14.3% vs 27.3%), gastrointestinal bleeding (0% in each group), or mortality rate (33.3% vs 29.3% vs 36.3%). Only 1 patient in the azoles group experienced LTLI with a new diagnosis of hepatocellular carcinoma 3 years after the index date.

TABLE 20. Exposure to antifungal treatment

CHARACTERISTICS	Total N=60	Micafungin n=20	Other echinocandins n=20	Azoles n=20	*P*
Reason for starting AF therapy					
Empirical therapy	24 (40.0)	10 (50.0)	9 (45.0)	5 (25.0)	0.23
Targeted therapy	36 (60.0)	10 (50.0)	11 (55.0)	15 (75.0)	
Length of AF treatment (median, range)	14.1 ± 8.0	12.3 ± 6.5	10.9 ± 5.6	19.2 ± 9.1	**<0.001**
Invasive fungal infection					
Bloodstream infection	20 (33.3)	5 (50.0)	6 (54.5)	9 (60.0)	0.88
Abdominal infection	5 (8.3)	2 (20.0)	0	3 (20)	0.13
Urinary tract	6 (10.0)	2 (20.0)	2 (18.9)	2 (13.3)	0.89
Lung	2 (3.3)	0	1 (9.1)	1 (6.7)	0.64
Other[&]	3 (5.0)	1 (10.0)	2 (18.9)	0	0.24
Septic shock	17 (28.3)	7 (35.0)	10 (50.0)	0	0.22
SOFA score (mean ± SD)	6.8 ± 3.2	6.5 ± 3.2	8.1 ± 2.8	5.8 ± 3.2	0.06

Development of short-term hepatotoxicity	6 (10.0)	2 (10.0)	2 (10.0)	2 (10.0)	1
In-hospital mortality	22 (36.6)	8 (40.0)	9 (45.0)	5 (25.0)	0.39

AF antifungal; **ICU** intensive care unit; **SOFA** sequential organ failure assessment; **SD** standard deviation;

Risk factors for liver injury

We performed a univariate analysis in order to identify potential risk factors for STLI, including the following variables: age, other underlying conditions, severity of liver disease, septic shock, baseline liver and renal function, and length of antifungal treatment. The only variables associated with STLI were presence of septic shock at the time of antifungal therapy (66.7% vs 24.1%, p=0.04) and higher MELD score (24.6 ± 8.6 vs 16.9 ± 6.8, p= 0.01) **(Table 21)**. However, differences were not significant for any variables in the multivariate analysis. Given that only 1 patient had LTLI, univariate analysis was not performed to identify risk factors for long-term liver complications.

Table 21. Univariate models predicting short-term liver toxicity

CHARACTERISTICS	No liver injury n=54	Hepatotoxicity n=6	*p*
Age, years (mean ± SD)	57.8 ± 14.8	61.5 ± 13.1	0.56
Male sex	42 (77.8)	3 (50.0)	0.15
Severity of liver disease			
Child B	32 (37.0)	3 (50)	0.68
Child C	22 (40.7)	3 (50)	0.68
Baseline Child-Pugh score	9.0 ± 1.4	10.0 ± 1.5	0.12
Baseline MELD score	16.9 ± 6.9	24.9 ± 8.5	**0.001**
Reason for starting AF therapy			
Empirical therapy	20 (37.0)	4 (66.7)	0.20
Targeted therapy	34 (63.0)	2 (33.3)	
Septic shock	13 (24.1)	4 (66.7)	**0.04**
Length of AF treatment	14.5 ± 8.2	10.5 ± 5.2	0.24
Micafungin treatment	18 (33.3)	2 (33.3)	1

SD standard deviation; **ICU** intensive care unit; **AF** antifungal

The incidence of STLI according to the baseline MELD score is reported in **Table 22**. When the baseline MELD score was > 20, the incidence of STLI was significantly higher in patients with azoles (2/3, 66.6%) than those treated with micafungin (1/9, 11.1%) or other ecchinocandins (1/10; 10%).The median time between the index date and the development of STLI was 7 days (range 3-14 days)

Table 22. Incidence of STLI according to baseline MELD score

MELD SCORE	Micafungin n=20	Other echinocandins n=20	Azoles n=20
<10	0/1 (0)	0/0	0/4
10-20	1/10 (10.0)	1/10 (10.0)	0/13
>20	1/9 (11.1)	1/10 (10.0)	2/3 (66.7)

DISCUSSION

To our knowledge, the present study is the first to show that, compared with other echinocandins or azoles, exposure to micafungin in patients with ESLD does not increase the risk of STLI and LTLI.

The incidence of IFI among patients with advanced liver disease has been reported to be as high as 10% [193, 194], with a mortality rate ranging from 56% to 98% [10, 174, 177, 195, 196]. In addition, liver disease has been found to be an

independent risk factor for mortality [10], possibly because of abnormalities of the immune system [178, 197-199]. This risk is proportional to the level of hepatic impairment [177, 200].

The choice of antifungals in patients with ESLD is limited by a number of factors including medical comorbidities, drug-drug interactions, and antifungal resistance [201], although the main factor limiting treatment is the hepatotoxicity of antifungal drugs [202]. Liposomal amphotericin B and azoles are both associated with a significant risk of hepatotoxicity (27%) [192, 202-204]. Moreover, liposomal amphotericin B has been associated with an increasing risk of nephrotoxicity and infusion-related reactions [205], whereas azoles have a limited spectrum of antifungal activity and can cause severe drug-drug interactions [206]. Thus, the use of alternative antifungal drugs for the treatment of IFIs in patients with pre-existing liver disease is of particular clinical relevance.

Echinocandins have an excellent safety profile and are promising agents for the treatment of IFI in patients with ESLD. Both anidulafungin and caspofungin have been studied for this indication, although information is mainly from patients with Child-Pugh A or B liver disease, and experience in Child-Pugh C disease is very limited [179-183].

Micafungin has a broad in vitro spectrum, potent in vivo activity, a favorable safety profile, and excellent bioavailability and is indicated for the treatment of invasive candidiasis [207, 208], esophageal candidiasis [209], and antifungal prophylaxis in patients with hematological disease [210].

Micafungin is generally safe in patients who do not have chronic liver disease, with no evidence of a greater risk of STLI than other antifungal drugs [207-210]. A systematic review and meta-analysis [192] showed that, an abnormal liver function test results during treatment with micafungin was observed in 3% of the patients with only 2.7% discontinuing treatment for hepatotoxicity. In a more recent study, no increased risk of short term liver injury was observed in comparison to other antifungals (fluconazole, caspofungin, voriconazole and amphotericin B) in a cohort of pediatric and adult patients without chronic renal and liver conditions who received micafungin; however micafungin was associated with a lower risk of renal dysfunction.

The evidences of hepatic toxicity in patients with chronic liver disease receiving micafungin are very limited but also generally reassuring [185, 186]. Nevertheless, the relative magnitude of short term liver risk compared to other antifungals in ESLD population is less clear. Our data shows that micafungin, in comparison to other echinocandins or azole therapy, did not incur a higher risk of hepatotoxicity in patients with ESLD. Indeed, groups had similar rate of STLI and a comparable change in transaminase level during therapy. More specifically, transaminase level remained stable or decreased during micafungin therapy and discontinuation of micafungin due to its hepatic adverse effects was required in one patient only. Moreover, in our study, in comparison to fluconazole, micafungin and the other echinocandins were more commonly

prescribed among patients with higher MELD score, which is by itself a risk factor for STLI.

As for LTLI, we found no major safety concerns relating the development of hepatic tumors during a follow-up period of more than one year. Preclinical data from animal studies, reported development of foci of alterated hepatocytes and hepatocellular tumors in rats treated with high doses of micafungin for prolonged periods. Interestingly, although similar studies have never been performed for anidulafungin [211] or caspofungin [212], comparable results were also observed in long term studies performed in animals receiving voriconazole [213] or fluconazole [214]. In the present study, we observed only one patient previously treated with azoles experiencing a new diagnosis of hepatocarcinoma during the follow- up period. We did not find evidence of liver tumors in any of the ESLD patients treated with micafungin, thus pointing to potential differences in tumor development between humans and rats. Our findings are consistent with the results from a pooled safety analysis including 3,028 patients treated with micafungin [191] and support the absence of post-marketing reports of hepatic adenoma or carcinoma related to micafungin, despite more than 1,000,000 patients worldwide have received the drug.

When we tried to analyze physician reasons for prescribing micafungin in ESLD patients, we could identify specific medical themes in only 5 out of 20 patients, consisting mainly in clinical failure with previous antifungals or drug-drug interactions. The lack of specific reasons to prescribe micafungin probably

reflects the fact that physician rely on safety and good tolerability of micafungin, as observed in previous studies [215].

Mayor limitations of this study include its retrospective design and the relatively small number of patients included. However, patients were identified after checking more than 2,000 patients in 6 large Spanish and Italian hospitals. Channeling bias is also likely since providers who are aware of the EMA warning would be expected to avoid prescription of micafungin in patients with a higher risk of developing short and long liver injury. Moreover, although we used a predefined definition for hepatic injury, a direct relationship between hepatotoxicity and antifungals exposure could be difficult to evaluate, especially in high risk group of patients in which other variables (comorbidities, other drugs, toxin etc etc) may have played a role leading to over- or underreporting toxicity in our analysis. Strengths of our study include its multicentric design and its optimal follow-up. To our knowledge this study is the first in –depth report on short and long term safety of micafungin in patients with ESLD and its comparison with other echinocandins and azoles.

In conclusion, the administration of micafungin therapy to patients with ESLD was safe and did not imply a higher risk of developing short nor long term liver injury

IX. SEXTO OBJETIVO:

El efecto engañoso del galactomanano sérico en pacientes hematológicos de alto riesgo que reciben profilaxis con micafungicina

Effect of serum galactomannan testing in high-risk hematology patients receiving prophylaxis with micafungin

INTRODUCTION

Invasive aspergillosis (IA) is a major cause of morbidity and mortality in patients with haematological malignancies and prolonged neutropenia and in patients undergoing hematopoietic stem cell transplantation (HSCT) [28, 216, 217].

Diagnosis of IA at an early stage of infection improves survival, as it enables prompt treatment [28]. Galactomannan (GM) is a component of the *Aspergillus* cell wall released during growth of hyphae that can be detected early in serum and in other samples from patients with IA; therefore, detection of GM is widely used as a screening test in high-risk patients [74, 75]. However, numerous causes of false-positive results have been identified in serum GM assays [218-228]. Furthermore, recent studies have shown that during prophylaxis with posaconazole, determination of serum GM remains useful only for diagnosis of symptomatic patients with clinical suspicion of IA, since results in asymptomatic patients were either negative or false-positive [76].

Micafungin has also been approved for prophylaxis in high-risk haematology patients and might be preferable to posaconazole in some situations because of its safety profile and the absence of significant drug interactions [229, 230]. To the best of our knowledge, no studies have investigated the diagnostic value of serum GM in high-risk haematology patients receiving prophylaxis with micafungin.

Therefore, the present study aimed to assess the diagnostic performance of serum GM in haematology patients during high risk episodes for IA, receiving

prophylaxis with micafungin and to describe the rate of breakthrough IA in this population.

MATERIALS AND METHODS

Study setting and population

This retrospective study was performed at Hospital General Universitario Gregorio Marañón from January 2010 to December 2015. All hospitalized high-risk hematology patients receiving prophylaxis with micafungin (standard dose of 50 mg daily) [231, 232] were included only if they underwent sGM testing either as a screening tool ("surveillance GM") or as a diagnostic test ("diagnostic GM"). The local ethics committee approved the study.

Definitions

Patients considered to be at high risk of IA were as follows: 1) patients with acute myeloid leukemia or myelodysplastic syndrome with neutropenia after remission-induction or consolidation chemotherapy; 2) patients who had received an allogeneic HSCT during the early neutropenic phase until engraftment and up to day 100 after transplant; and 3) HSCT recipients who had had graft-versus-host disease requiring high doses of systemic prednisone therapy (>1 mg/kg/day).

IA was defined according to the revised criteria proposed by the EORTC/MSG [74]. During the high-risk episode, local protocols were used for the

management of neutropenic fever or clinical suspicion of IA, but the choice to perform additional testing or to replace prophylaxis with empirical antifungal therapy was made by the attending physician.

According to previous methodology [76].and to sGM results, episodes were classified as follows: **true-positive** (positive sGM result in the context of breakthrough IA), **false-positive** (positive sGM result in a patient who remained on prophylaxis with micafungin and had no evidence of IA), **true-negative** (episodes with all consecutive negative sGM tests results and no other diagnostic features of IA), **false-negative** (negative sGM test results in the context of breakthrough IA), and **non-evaluable** (patients receiving antifungal treatment for different reasons).

sGM tests were considered positive when an optical density index of ≥0.7 was reached in 1 sample or ≥0.5 in 2 consecutive samples. The sGM test was considered to be a screening tool for IA (surveillance sGM) when it was systematically performed twice weekly in asymptomatic, afebrile patients. Conversely, an sGM test was considered a diagnostic tool for IA (diagnostic sGM) when it was triggered by 1 of the following findings: 1) neutropenic fever despite 5 days of therapy with a broad-spectrum antibiotic; 2) clinical or radiological features suggestive of IA; 3) isolation of molds from respiratory specimens. *Aspegillus* PCR and fungal cultures were performed as previously described [233, 234]

Data analysis

We analysed sensitivity, specificity, positive predictive value (PPV), and negative predictive value (NPP) of sGM test in high risk haematological patients using SPSS 18 statics.

RESULTS

Study populations

The study population included 149 patients (mean age, 43.5 years; men, 60.4%) who had experienced 208 high-risk episodes of IA. The reasons for antifungal prophylaxis in the 208 high-risk episodes are reported in **Table 23**. The median duration of micafungin prophylaxis was 23 days [range, 7-254].

Table 23. Reason for antifungal prophylaxis in 208 high-risk episodes

Characteristics	N (%)	%
AML/MDS chemotherapy	**40**	**19.3**
Induction	23 (57.5)	11.1
Consolidation	10 (25)	4.8
Salvage	7 (17.5)	3.4
Allogenic HSCT	**129**	**62.0**
Myeloablative	55 (42.6)	26.4
Reduced intensity	31 (24.0)	14.9
Related donor	84 (65.1)	40.4
Unrelated donor	24 (18.6)	11.5
Cord blood	10 (7.8)	4.8
Dual	2 (0.2)	1
Graft-versus-host disease	**39**	**18.7**
Acute	35 (89.7)	16.8
Chronic	4 (10.3)	1.9
Skin	2 (5.1)	1
Gastrointestinal	29 (82.9)	13.9
Liver	6 (15.4)	2.9
Lung	1 (2.1)	0.5
Other	1 (2.1)	0.5

Abbreviations: AML, acute myeloid leukemia; **MDS**, myelodysplastic syndrome; **HSCT**, hematopoietic stem cell transplant.

Overall, 62 episodes (29.7%) were considered **non-evaluable**, because micafungin had been switched to another antifungal treatment for the following reasons: a) febrile neutropenia without microbiological confirmation (58 episodes); b) breakthrough invasive fungal infections (IFI) in 3 episodes (*Trichosporon asahii, C. albicans*, and *Rhizosporum* spp); and c) side effects requiring discontinuation of micafungin (1 episode).

Of the **146 evaluable episodes**, 4 had a positive sGM result in the context of probable IA and were therefore considered **true-positive**. Accordingly, the incidence of breakthrough IA under prophylaxis with micafungin was 2.7%. The clinical characteristics of these 4 patients are summarized in **Table 24.** Mortality was 25%.

The GM test yielded **true-negative results** in most episodes (111/146; 76%). Mortality in this group was 3.6% (5 patients), with no deaths related to IFI.

In 31 episodes (21.2%), sGM results were positive in the absence of IA (**false-positive results**), with an OD value ranging from 0.51 to 4.64. All but 1 false-positive episodes were observed in surveillance sGM. None of these patients were diagnosed with IA, and specific treatment was not administered. Mortality in this group was 16.1% (5 patients); 3 patients died from bacterial infections and 2 from cardiologic complications. A potential cause of the false-positive result was obtained in 29/31 patients: platelet transfusion was the most common cause (74.1%), followed by total parenteral nutrition (20.3%) and

administration of piperacillin/tazobactam (5.6%). No **false-negative** GM results were detected.

Table 24. Cases of breakthrough invasive aspergillosis in patients receiving prophylaxis with micafungin (all with true-positive galactomannan test results)

Characteristics	Patient 1	Patient 2	Patient 3	Patient 4
Age (years)	30	33	11	46
Sex	Male	Male	Male	Male
High-risk episode	Chronic GVHD	AML-salvage chemotherapy	Cord blood allo-HSCT	Acute GVHD
Days of prophylaxis before the diagnosis of IA	13	5	14	28
Location of IA	Paravertebral	Paranasal sinuses	Pulmonary	Pulmonary
Positive GM in serum	Yes	Yes	Yes	Yes
Initial value	0.93	1.30	0.99	1.75
Symptoms	Lumbar pain and persistent fever	Acute sinus pain and persistent fever	Asymptomatic	Persistent fever
Fungal culture and PCR	Paravertebral mass: Culture-negative Calcofluor stain: septate hyphae Panfungal PCR: *Aspergillus sp*	Negative	Negative	Negative

Histology	Negative (no hyphae)	No biopsy	No biopsy	No biopsy
Image findings	T11 paravertebral abscess in MRI	Sinusitis in CT scan	Bilateral multifocal nodules with halo sign in chest CT scan	Bilateral multifocal nodules with halo sign in chest CT scan
Length of hospitalization (days)	85	54	40	190
Outcome	Alive	Alive	Alive	Dead

Abbreviations: Allo-CT, allogeneic hematopoietic cell transplant; **AML**, acute myeloid leukemia; **BAL**, bronchoalveolar lavage; **CT**, computed tomography; **MRI**, magnetic resonance imaging; **GM**, galactomannan; **GVHD**, graft versus host disease; **IA**, invasive aspergillosis

Diagnostic performance of sGM

Diagnostic performance of sGM was calculated based on the 146 evaluable episodes (**Table 25**). Overall, the test had a sensitivity of 100% and a specificity of 78.1%. When sGM was used for surveillance, the NPV was very high (100%), but the PPV was only 3.1%, and most cases were false-positive. However, the PPV increased to 75% when sGM was performed because of clinical suspicion of IA (diagnostic).

Table 25. Diagnosis performance of serum galactomannan in hematologic patients undergoing antifungal prophylaxis with micafungin

EVALUABLE EPISODES	
GM test results	146
True positive, No (%)	4 (2.7)
True negative, No (%)	111 (76)
False positive, No %	31 (21.2)
False negative, No %	0
Sensitivity, %	100
Specificity, %	78.1
Scenario 1: Surveillance sGM test	
Negative predictive value, %	100
Positive predictive value, %	3.1
Scenario 2: Diagnostic driven sGM test	
Negative predictive value, %	100
Positive predictive value, %	75

DISCUSSION

The results of the last study demonstrate that sGM screening in high-risk patients receiving prophylaxis with micafungin is an unsuitable diagnostic approach for IA because of its low PPV and high rate of false-positive results. However, sGM testing remains useful in the diagnosis of breakthrough IA in symptomatic patients receiving prophylaxis with micafungin.

During the last decade, the strategy of empirical antifungal therapy in high-risk hematology patients has been based on the performance of serial detection of sGM antigen, which has enabled early diagnosis, substantial sparing of unnecessary antifungal treatment, and reduced toxicity and costs [235]. However, the value of the sGM test for the diagnosis of IA depends on the pre-test probability of disease, which considerably affects performance in terms of sensitivity and specificity [236, 237]. In recent years, the implementation of anti-mold prophylaxis in high-risk hematology patients has significantly reduced the incidence of IFI in such populations [238] and impacted on the sensitivity of the sGM test. Duarte et al [76] recently published the results of a prospective study on the reliability of sGM for early detection of IA in 262 high-risk patients receiving prophylaxis with posaconazole. The overall incidence of IA in their population was 1.9%, whereas the PPV of sGM was only 11.8% in tests performed as pre-emptive surveillance, although this value improved to 89.6% when tests were performed in the presence of clinical suspicion of IFI.

In recent years, the number of hematology patients receiving prophylaxis with micafungin has increased dramatically, mainly owing to the drug's efficacy [239], scarce interactions with other drugs, and lack of inter-individual variations. The current study overcomes the limitations of Duarte et al [76] by including patients receiving micafungin, thus providing additional information on the diagnostic value of sGM. In almost all cases in our series in which sGM tests were useful for diagnosis, they were indicated because of clinical suspicion of IFI. Accordingly, the PPV of the sGM test was only 3% as a surveillance tool, although it rose to 75% when the test was performed as part of a diagnostic strategy.

Our study confirms that a tailored diagnostic approach is useful and safe for diagnosing IA in high-risk patients receiving prophylaxis with micafungin [76]. As for outcome, none of the 3 symptomatic patients who developed IA died and all had radiological and clinical features suggestive of IFI before performance of the sGM test.

Our study is subject to a series of limitations. First, it is a retrospective study and includes a relatively low number of patients with a diagnosis of proven or probable IA. However, this number is comparable to that reported in previous studies [76]. Second, we included patients who received antifungal prophylaxis for at least 4 days. Although this timeframe could be considered excessively short, we decided to adopt these criteria in order to compare our

data with those of previous studies [240]. Finally, we used sGM to classify disease, thus its diagnostic performance could be overestimated.

In conclusion, we found that sGM-based surveillance of asymptomatic patients receiving prophylaxis with micafungin should not be performed, because nearly all results are either negative or false-positive. However, sGM testing remains useful in the diagnosis of breakthrough IA in symptomatic patients receiving prophylaxis.

X.DISCUSION INTEGRADORA

DISCUSION INTEGRADORA

Dado que se trata de una discusión general y que la discusión en los aspectos particulares se ha hecho en cada uno de los artículos, he preferido ofrecer en esta discusión integradora una mera especulación sobre el futuro del campo de la micología médica, que refleje tanto los resultados de mis artículos como la visión y los deseos que, tras mi entrenamiento, tengo para el futuro en este campo.

De hecho, imaginando la micología del 2050, es imposible no plantearse una serie de preguntas de las que he querido discutir en este apartado de la tesis. Claramente es muy difícil ofrecer una visión que prediga perfectamente al futuro, ya que es imposible considerar todas las variables inesperadas que inevitablemente influyeran sobre la epidemiologia y el manejo de las IFI.

Aproximación futura: Progreso en términos diagnósticos

Como se ha destacado en los dos primeros trabajos de esta tesis, la introducción en los últimos años de herramientas diagnósticas como el T2MR *Candida* constituyen una verdadera revolución diagnóstica en el campo de la micología médica. Esta revolución está llevando al abandono del diagnóstico microbiológico basado en cultivos tradicionales, en favor de nuevas técnicas rápidas sobre muestras directas del paciente, que son capaces de proporcionar información clínica relevante en pocas horas.

De hecho, se espera que en los próximos años se produzca una disminución progresiva de los diagnósticos obtenidos por cultivos tradicionales, que irán perdiendo importancia en el tratamiento diario de los pacientes. Es probable que las técnicas estándar no desaparezcan por completo, y que su utilidad clínica se limite en un futuro a estudios epidemiológicos.

Las nuevas técnicas diagnósticas, serán menos invasivas y más rápidas, y permitirán identificar un hongo a nivel de especie en pocas horas, ofreciendo información sobre resistencia antifúngica e incluso permitirán el genotipado. Se espera que estas nuevas técnicas permitan resolver definitivamente el mayor problema aún abierto en la micología médica, la diferenciación entre colonización e infección.

Parte de este progreso ya se ha logrado con la introducción de MALDI-tof, que en algunas especies bacterianas ya puede proporcionar información sobre el perfil de resistencia. Además, la introducción de la nariz electrónica permitirá el análisis a pie de cama del paciente de partículas volátiles y permitirá evaluar la presencia del hongo de forma rápida y no invasiva.

Asistiremos dentro de algunos años a la introducción de nuevas técnicas de imagen que permitan identificar con mayor precisión la localización anatómica de la infección fúngica. Estas técnicas se basaran probablemente en el uso de anticuerpos específicos capaces de identificar la presencia de hifas fúngicas en diferentes tejidos, permitiendo así un diagnóstico "radiológico" de la IFI. Otra ventaja hipotética de este tipo de técnicas, será la capacidad de

cuantificar la carga fúngica presente en los distintos sitios anatómicos, lo que serviría para modular el tipo y duración del tratamiento antifúngico.

Es de esperar que en un futuro no muy lejano, estas nuevas técnicas de diagnóstico nos permitan conocer mejor la patogenia de las IFIs, calcular el tiempo preciso de incubación, pudiendo así finalmente diferenciar las infecciones adquiridas en la comunidad de las nosocomiales.

Aproximación futura: Progreso en términos terapéuticos

Con la mejora de las técnicas diagnósticas y la mejor identificación de los pacientes con alto riesgo de desarrollar una IFI, se anticipa cada vez más el inicio precoz del tratamiento antifúngico dirigido, con la consiguiente desaparición de la profilaxis y los tratamientos empíricos, que en 30 años serán un viejo recuerdo del pasado.

Con la ayuda de la farmacogenética y de la secuenciación masiva del genoma, utilizaremos cada vez más tratamientos antifúngicos "a medida" para cada paciente y para el patógeno específico. La farmacogenética nos permitirá identificar a los pacientes con riesgo de desarrollar efectos adversos relacionados con el uso de ciertos fármacos (hepatotoxicidad, nefrotoxicidad). Mientras que con la secuenciación masiva del genoma, conoceremos mejor los perfiles de resistencia de los microorganismos de una forma más rápida, y podremos elegir mejor el fármaco con mayor actividad antifúngica.

Como hemos demostrado en el objetivo número 5 de esta tesis, la dosis de los fármacos ya no será la misma dosis estándar para todos los pacientes, sino una dosis específica individualizada para cada paciente que maximizará la actividad terapéutica al reducir los efectos secundarios. Todo esto también evitará el uso de terapias combinadas que conllevan mayor toxicidad, en favor de tratamientos antifúngicos más simples.

También utilizaremos nuevas moléculas antifúngicas, dirigidas a nuevas dianas, diferentes de la pared celular de los hongos y la forma en que se administran los medicamentos también cambiará. La administración parenteral pasará de contar con fármacos que se administran una vez al día a fármacos que se administran una vez por semana, permitiendo dar de alta precozmente a los pacientes y disminuyendo los días de uso de catéteres venosos. Además, muy probablemente surjan antifúngicos con una buena biodisponibilidad vía oral que sustituyan a los actuales y que no requieran de una estricta monitorización serológica de sus niveles. Otra posibilidad es que se desarrollen nuevos antifúngicos que sean capaces de llegar selectivamente a ciertos sitios anatómicos. Esto significa, por ejemplo, que la aspergilosis podrá ser tratada en el futuro mediante la instilación de antifúngicos que tengan selectividad directa a nivel de los segmentos pulmonares afectados por la infección.

Y quién sabe, si en un futuro, con inminente entrada de anticuerpos monoclonales en el campo de las enfermedades infecciosas, puedan surgir terapias específicas para las principales IFIs. De hecho, hasta ahora hemos

utilizado moléculas que actúan directamente contra la pared del hongo, mientras que el uso de nuevas inmunoterapias, quizás mejor toleradas y menos tóxicas, puedan resolver el problema inmunológico que ha favorecido la aparición de la infección.

Además, con la ayuda de las técnicas mencionadas anteriormente, finalmente tendremos la oportunidad de decidir una duración óptima específica para cada paciente, con lo que se evitará el gran problema de la duración excesiva de los tratamientos antifúngicos.

Aproximación futura: Progreso en profilaxis

En mi opinión, al igual que el tratamiento empírico, el número de pacientes que reciben tratamiento profiláctico también disminuirá progresivamente hasta que desaparezca por completo.

Un gran avance vendrá del análisis de factores genéticos capaces de predisponer al desarrollo de las infecciones fúngicas. En el futuro podremos identificar mejor una subpoblación de pacientes con factores de riesgo para desarrollar una IFI que realmente necesite recibir tratamiento profiláctico debido a una mayor predisposición genética.

Además, las estrategias de prevención se centrarán principalmente en el medio ambiente. Mediante el uso de nuevos dispositivos, posiblemente portátiles, será posible minimizar la carga fúngica tanto a nivel del aire como en el espacio circundante. El paciente hematológico podrá pasear libremente

dentro y fuera del hospital sin tener que estar "segregado" en habitaciones con filtros HEPA y ya no observaremos brotes con transmisión horizontal de infecciones por *Candida* en unidades de cuidados intensivos.

Finalmente, el estudio de la microbiota humana puede dar como resultado un gran aporte. Hasta ahora, de hecho, se ha prestado mucha atención a las bacterias y poca a los hongos. "Por lo tanto, es posible que en el futuro el análisis de la microbiota humana permita identificar exactamente a ese grupo de pacientes en riesgo de desarrollar una infección fúngica endógena y eliminar la colonización por levaduras intestinal por levaduras resistentes a antifúngicos".

Aproximación futura: cambios epidemiológicos

A pesar de los avances en la prevención, es muy probable que el número absoluto de casos de infección fúngica aumente en los próximos 50 años. Se producirá un cambio progresivo tanto en términos epidemiológicos como en términos de poblaciones en riesgo. De hecho, los casos de infecciones por *Candida* y *Aspergillus* en las poblaciones clásicamente afectadas por estas infecciones disminuirán progresivamente y a medida que mejoren las técnicas de diagnóstico podremos identificar otros grupos de población en riesgo (por ejemplo, pacientes con nuevos fármacos inmunológicos –anticuerpos monoclonales-, pacientes que reciben nuevos

fármacos de quimioterapia, etc.) que serán susceptibles de desarrollar formas subagudas de infecciones fúngicas.

Al mejorar también la identificación del paciente en riesgo de desarrollar una IFI, es probable que las causas más frecuentes de infecciones micóticas sean un problema marginal en el futuro. Por otro lado, con la progresión de la medicina, con la edad avanzada de los pacientes que serán cada vez más portadores de nuevos dispositivos y que recibirán nuevos fármacos, es posible que nuevos agentes etiológicos, actualmente inofensivos y totalmente desconocidos para nosotros desde el punto de vista de la patología humana, se conviertan en el futuro en las principales causas de infecciones fúngicas emergentes en los hospitales.

A partir de estas consideraciones es fácil entender cómo el papel del especialista en infecciones y del microbiólogo clínico sigue siendo fundamental.

XI.CONCLUSIONES

CONCLUSIONES

1. El 30,0% de los pacientes con candidemia presentan una forma “complicada”, definida como un episodio que causa metástasis sépticas o que es responsable de la muerte del paciente. Ningún elemento clínico es capaz de diferenciar entre pacientes con candidemia complicada y no complicada.
2. En comparación con los hemocultivos de control o el 1→3 β-D-glucano, la realización de T2MR de control en pacientes con candidemia podría predecir mejor a los pacientes con candidemia complicada, incluso aquellos con hemocultivos de control negativos.
3. Entre toda la población de pacientes que recibieron un tratamiento antifúngico empírico, tan solo el 28% de ellos tuvo una mala evolución clínica (candidiasis invasiva probada o muerte en los primeros 7 días).
4. En pacientes con sospecha de candidiasis invasiva en los que se ha iniciado un tratamiento antifúngico empírico, la determinación precoz del T2MR en combinación con los cultivos tradicionales puede, con una alta capacidad discriminatoria, identificar a los pacientes con mala evolución clínica.
5. Esta estrategia puede permitir de identificar a los pacientes que realmente pueden beneficiarse de un tratamiento antifúngico más largo.

6. En un hospital con gran experiencia en políticas de antifúngicos, la implementación sistemática de un “check-list” que incluya recomendaciones estructuradas, dirigidas a garantizar la adherencia a las guías clínicas, es capaz de mejorar el pronóstico de los pacientes con candidemia disminuyendo de forma significativa la mortalidad a 14 y 30 días.
7. En un grupo no seleccionado de pacientes hospitalizados que reciben triazoles o equinocandinas, existe una correlación débil entre la dosificación antifúngica estándar, basada en las guías, y las concentraciones séricas adecuadas del fármaco, con una gran proporción de pacientes fuera del rango terapéutico (~ 30%).
8. Una concentración sérica adecuada del antifúngico (y non una dosis adecuada según las guías) parece predecir mejor el desenlace clínico de los pacientes, lo que aconseja la realización de TDM de antifúngicos de forma rutinaria.
9. En el estudio multicentrico retrospectivo que incluya una población de pacientes con hepatopatía grave, la frecuencia de daño hepático agudo debido a antifungicos fue del 10%.
10. La incidencia de daño hepático agudo fue igual entre los pacientes que recibieron azoles (10%), micafungina (10%) u otras candinas (10%)

11. Durante el período de seguimiento, sólo 1 paciente, que previamente había recibido tratamiento con azoles, desarrolló un hepatocarcinoma.
12. La tasa de aspergilosis invasora de brecha en una población de pacientes hematológicos que reciben profilaxis con micafungina es tan solo del 2.7%.
13. En este grupo de paciente, la determinación del galactomannano sérico como prueba de depistaje de aspergilosis invasora es un enfoque diagnóstico inadecuado debido a su bajo valor predictivo positivo y la alta tasa de resultados falsos-positivos. Sin embargo, la prueba del galactomannano sérico sigue siendo útil como test diagnóstico de aspergilosis invasora en pacientes con síntomas clínicos.

XII.PRODUCCIÓN CIENTÍFICA

PRODUCCIÓN CIENTÍFICA

Esta tesis doctoral, ha permitido la realización de seis artículos científicos. De estos, tres han sido ya publicados en revistas científicas internacionales de alto factor de impacto y tres están actualmente pendiente de revisión.

- **ARTICULOS PUBLICADOS**

1. Munoz, P., A. Vena, M. Machado, F. Gioia, M. C. Martinez-Jimenez, E. Gomez, J. Origuen, M. A. Orellana, F. Lopez-Medrano, M. Fernandez-Ruiz, P. Merino, F. Gonzalez-Romo, I. Frias, M. J. Perez-Granda, J. M. Aguado, J. Fortun, and E. Bouza. 2018. T2Candida MR as a predictor of outcome in patients with suspected invasive candidiasis starting empirical antifungal treatment: a prospective pilot study. J Antimicrob Chemother 73:iv6-iv12. 20.
2. Munoz, P., A. Vena, M. Machado, M. C. Martinez-Jimenez, F. Gioia, E. Gomez, J. Origuen, M. A. Orellana, F. Lopez-Medrano, M. J. Perez-Granda, J. M. Aguado, J. Fortun, and E. Bouza. 2018. T2MR contributes to the very early diagnosis of complicated candidaemia. A prospective study. J Antimicrob Chemother 73:iv13-iv19.

3. Vena, A., E. Bouza, A. Alvarez-Uria, J. Gayoso, P. Martin-Rabadan, F. Cajuste, J. Guinea, J. Gomez Castella, R. Alonso, and P. Munoz. 2017. The misleading effect of serum galactomannan testing in high-risk haematology patients receiving prophylaxis with micafungin. Clin Microbiol Infect 23:1000 e1-1000 e4.

- **ARTÍCULOS PENDIENTES DE REVISIÓN:**

1. Vena A, Muñoz P, Mateos M., Guinea J, Galar A. Pea F, Alvarez-Uria A., Escribano P., Bouza E. Therapeutic drug monitoring of antifungal drugs: another tool to improve patient outcome? Under review Transplant Infectious Disease
2. Vena A., Bouza E, Bassetti M, Menichetti F, Merelli M., Grau S., Fortun J, Sánchez MI., Aguado J.M; Merino P., Bonache F, Muñoz P. Comparison of the safety and tolerance profile of micafungin with that of other echinocandins and azoles in patients with pre-existing Child-Pugh B or C liver disease. Under review Expert Opinion on Drug safety.
3. Vena A; Bouza E., Corisco R, Machado M, Valerio M, Sanchez C., Muñoz P. Efficacy of a “check list” intervention bundle on the clinical outcome of patients with *Candida* bloodstream infections. Under review Open Forum Infectious Disease.

Además, esta tesis doctoral ha servido de base para el inicio de una línea de investigación con la producción científica de *25 artículos* hasta el momento, tanto dentro de nuestro grupo de trabajo como en cooperaciones internacionales

1. Munoz P, **Vena A**, Ceron I, Valerio M, Palomo J, Guinea J, Escribano P, Martinez-Selles M, Bouza E. Invasive pulmonary aspergillosis in heart transplant recipients: Two radiologic patterns with a different prognosis. **J Heart Lung Transplant**. 2014;33:1034-1040
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4. Munoz P, Valerio M, **Vena A**, Bouza E, Posteraro B, Lass-Florl C, Sanguinetti M. Advances in the management of fungal infections. **Mycoses**. 2015;58 Suppl 2:1

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6. Fortun J, Muriel A, Martin-Davila P, Montejo M, Len O, Torre-Cisneros J, Carratala J, Munoz P, Farinas C, Moreno A, Fresco G, Goikoetxea J, Gavalda J, Pozo JC, Bodro M, **Vena A**, Casafont F, Cervera C, Silva JT, Aguado JM. Caspofungin versus fluconazole as prophylaxis of invasive fungal infection in high-risk liver transplantation recipients: A propensity score analysis. **Liver Transpl.** 2016;22:427-435

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8. Martinez-Jimenez MC, Munoz P, Valerio M, **Vena A**, Guinea J, Bouza E. Combination of candida biomarkers in patients receiving empirical antifungal therapy in a spanish tertiary hospital: A potential role in

reducing the duration of treatment. **J Antimicrob Chemother**. 2016;71:2679

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Por último, me gustaría señalar otras tres aportaciones científicas al campo de las enfermedades infecciosas que he tenido el placer de dirigir a lo largo de los años de mi doctorado.

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XIII. BIBLIOGRAFÍA

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XIV. ANEXO 1

Table S1. Clinical characteristics of the 14 patients with poor outcome.

Age /Sex	Main underlying disease	Surgery; type	Septic shock	CVC	Type of IC	*Candida* sp	Length of therapy	Early Mortality	Day 0			Day 2			Day 4		
									CAGTA	BDG	T2	CAGTA	BDG	T2	CAGTA	BDG	T2
52/F	Gastrointestinal disease	Eventroplasty	Yes	Yes	Secondary Peritonitis	*C. albicans*	24	No	IND	-	-	IND	+	-	IND	+	-
55/M	Oesophagus cancer	Oesophagostomy	No	Yes	Pleural empyema	*C. albicans*	18	No	+	+	-	+	-	-	+	-	-
76/M	Cholangiocarcinoma	Cephalic duodenopancreatectomy	Yes	Yes	Candidemia secondary to intra-abdominal infection	*C. parapsilosis*	23	No	-	+	**+**	-	+	-	-	+	-
67/M	Gastric cancer	Gastrectomy with anastomotic dehiscence	Yes	Yes	Secondary Peritonitis	*C. glabrata*	28	No	-	-	-	-	+	-	-	-	-
67/M	Colorectal cancer	Abdominoperineal resection	No	No	Peritonitis	*C. albicans*	13	No			-	-	-	-	-	-	-
41/F	Ovarian cancer	Ovarian-uterus resection	No	No	Peritonitis	*C. albicans*	10	No	+	-	INV	+	-	INV	+	-	**+**
57/F	End stage liver cirrhosis	Liver transplantation	No	No	Peritonitis	*C. albicans*	7	Yes	-	+	**+**	-	+	+	-	+	-
66/M	Acute myocardial infarction requiring ECMO	No surgery	Yes	Yes	No definitive diagnosis of IC		3	Yes	+	+	+						
83/F	Gastrointestinal disease	Open inguinal hernia repair	Yes	Yes	No definitive diagnosis of IC	-	4	Yes	+	-	-	-	-	-			

65/M	Cardiovascular disease, Liver cirrhosis Intestinal ischemia	Laparotomy	Yes	Yes	No definitive diagnosis of IC	-	4	Yes	-	+	-	-	+	-			
79/F	Chronic renal failure	Hemicolectomy	Yes	Yes	No definitive diagnosis of IC	-	7	Yes	-	-	+	-	-	+			
77/M	Cardiovascular disease	Open inguinal hernia repair	Yes	Yes	No definitive diagnosis of IC	-	4	Yes	IND	+	-						
76/F	No underlying condition intestinal perforation	Ileocolic resection	Yes	Yes	No definitive diagnosis of IC	-	2	Yes	-	+	+	-	+	-			
80/M	Colorectal cancer	Hemicolectomy	Yes	Yes	No definitive diagnosis of IC	-	5	Yes	-	-	-	-	+	-	-	+	-

CVC Central Venous Catheter; **IC** Invasive Candidiasis; **BC** Blood Culture; **F** Female; **CT** Chemotherapy; **ECMO** Extracorporeal Membrane Oxygenation**; IND** Indeterminate; **INV** Invalid result;

Supplementary Material 2: ICD codes utilized to identify patients with pre-existing liver disease:

570, 571.1, 571.2, 571.3, 571.40, 571.41, 571.42, 571.42, 571.49, 571.5, 571.6, 571.8, 571.9
155.0, 155.1, 155.2
671.3
99.2

XV.RESUMEN

RESUMEN

INTRODUCCIÓN

En los últimos años, las infección fúngicas invasoras (IFI) han adquirido un protagonismo especial en los hospitales modernos, debido fundamentalmente a su elevada tasa de morbilidad y mortalidad y a su elevado consumo de recursos tanto para su prevención como para su manejo. Además, estas infecciones constituyen un reto concreto y continuo para los clínicos ya que existen muchas incertidumbres que afectan aspectos diagnósticos, terapéuticos y preventivos.

OBEJCTIVOS

Con esta tesis pretendemos aclarar una serie de dudas relacionadas con el diagnóstico y manejo de las IFI, que todavía no han sido resueltas por la comunidad científica. En concreto nos hemos planteado los siguientes objetivos:

- **Primer objetivo:** Describir la incidencia, factores clínicos predisponentes y la evolución de los pacientes con candidemia complicada. Evaluar si en comparación con los hemocultivos tradicionales y el (1→3)-beta-D-glucano, la determinación seriada de T2MR es capaz de discriminar entre candidemia complicada y no complicada.
- **Segundo objetivo:** Evaluar la utilidad clínica de los biomarcadores de candidiasis invasiva y del T2MR usados prospectivamente en pacientes con sospecha de candidiasis invasiva que reciben tratamiento empírico para la retirada precoz de los tratamientos antifúngicos innecesarios.

- **Tercer objetivo:** Describir en un hospital terciario con gran experiencia en política de antifúngicos, el impacto clínico que puede tener la aplicación sistemática de un paquete compuesto por medidas diagnósticas y terapéuticas sobre la morbi-mortalidad de los pacientes con candidemia.
- **Cuarto objetivo:** Definir si existe asociación entre la dosis de fármacos antifungicos y la concentración sérica adecuada en un grupo no seleccionado de pacientes hospitalizados. Evaluar el impacto clínico de una dosis inadecuada o concentraciones séricas inadecuadas en el desenlace clínico de los pacientes con infección fúngica invasiva.
- **Quinto objetivo:** Evaluar la eficacia, tolerabilidad y seguridad del tratamiento antifúngico con micafungina en pacientes con insuficiencia hepática severa. Definir si existe asociación entre exposición a micafungina y desarrollo de neoplasia hepática a largo plazo.
- **Sexto objetivo:** Investigar la rentabilidad diagnostica del galactomannano sérico en pacientes hematológicos de alto riesgo que reciben profilaxis con micafungina. Describir la tasa de aspergilosis invasora de brecha en esta población.

RESULTADOS

Primer objetivo: Desde enero hasta junio del 2017, fueron reclutados 30 pacientes con candidemia. De ellos, 9 (30%) desarrollaron una forma complicada de candidemia. La capacidad de predecir episodios de candidemia complicadas, asociados a los resultados persistentemente positivos en hemocultivos, T2

Candida Magnetic resonance (T2MR) y 1-3 β- D-glucano (BDG) fueron respectivamente: Sensibilidad (44,4%, 100%, 100%); Especificidad (76,1%, 76,1%, 38.9%); VPP (44,4%,64,2%, 40.9%) y VPN (76,1%; 100%, 100%). En el análisis multivariado, tener un T2MR positivo dentro de los primeros 5 días se asoció con un riesgo de casi 37 veces mayor de desarrollar una candidemia complicada.

Segundo objetivo: En el segundo estudio se incluyeron 49 pacientes con sospecha de candidiasis invasora. De estos, 14 (29%) tuvieron un mala evolución clínica. (7 murieron dentro de los primeros 7 días del tratamiento antifúngico, mientras que 7 terminaron con un diagnóstico de candidiasis invasora). Los resultados del Candida albicans germ tuve antibodies (CAGTA) [3/14 (21,4%) versus 8/35 (22,9%), p = 1] y del BDG [8/14 (57,1%) versus 17/35 (62,9%), p = 0,75] fueron similares en pacientes con mala y buena evolución. Por el contrario, un T2MR positivo se asoció con un mayor riesgo de mal pronóstico [5/14 (35,7%) versus 0/35 (0,0%)p=0,0001]. La especificidad y el VPP de un T2MR positivo para predecir el mal pronóstico fueron del 100%, con un VPN del 79,6%. Después de combinar los biomarcadores y el T2MR con los cultivos estándar, la combinación de T2MR y el cultivo estándar mostró una alta capacidad para discriminar a los pacientes con mala evolución de aquellos con una buena evolución clínica.

Tercero objetivo: En el tercero estudio se incluyeron 109 pacientes con candidemia, 56 en el período de pre-intervención y 53 en el período de post-intervención. En general, el cumplimiento del paquete de medidas de candidemia mejoró significativamente entre el período post-intervención (27/56, [48.2%] vs

43/53, [81.1%]; p=0.01). Además, en el análisis multivariado, la aplicación del paquete de medidas en el periodo post-intervención, tuvo un impacto favorable sobre la mortalidad a los 14 días (HR 0,08; IC del 95%: 0,01 a 0,45; p = 0,02) y la mortalidad a los 30 días (HR 0,40; IC del 95%: 0,18 a 0,89; p = 0,02).

Cuarto objetivo: Durante el período de estudio, 84 pacientes (65,4% hombres, 59,6 años) recibieron antifúngicos como profilaxis (40,4%), tratamiento dirigido (31,0%) y terapia empírica (28,6%). El fármaco más frecuentemente utilizado fue micafungina (28/84; 33,3%), seguido de fluconazol (23/84; 27,4%), voriconazol (15/84, 17,9%), anidulafungina (8/84, 9,5%), posaconazol (7 / 84, 8,3%) y caspofungina (3/84, 3,6%). Se observó una considerable variabilidad interindividual para todos los antifúngicos, con una gran proporción de pacientes (64,3%) que no alcanzaron las concentraciones séricas adecuadas a pesar de recibir una dosis antifúngica adecuada. Alcanzar el nivel sérico antifúngico adecuado se asoció significativamente con un resultado clínico favorable (OR = 0,02; IC del 95%: 0,01-0,64; p = 0,03), mientras que la administración de una dosis antifúngica adecuada no se asoció.

Quinto objetivo: Veinte de 2.335 (0,85%) pacientes con enfermedad hepática grado B o C de Child-Pugh recibieron micafungina. La frecuencia de daño hepático agudo debido a los antifungicos fue del 10% con ninguna diferencia entre pacientes que recibieron micafungina, otras candinas o azoles. La mayoría de los casos fueron asintomáticos y las antifungicos tuvieron que cambiarse a otra clase en solo 2 pacientes (1 micafungina y 1 azol). Durante el período de seguimiento,

se observó el desarrollo de hepatocarcinoma en solo 1 paciente, que previamente había recibido tratamiento con azoles.

Sexto objetivo: Entre los 146 pacientes hematologicos de alto riesgo evaluables, el galactomannano fue considerado como verdadero positivo en 4 pacientes, en los que se diagnosticó una probable AI de brecha (incidencia de AI de brecha, 2,7%); 111 episodios de alto riesgo (76%) se consideraron verdaderos negativos y 31 (21,2%) falsos positivos. No se detectaron resultados falsos negativos. Todos menos uno de los resultados falsos positivos del galactomanano se obtuvieron en pacientes en los que la prueba se pidió como depistaje de la infección. Los valores predictivos predictivos positivos y negativos del galactomannano (GM) sérico como prueba de depistaje o diagnóstico fueron 3,1% y 100% y 75% y 100%, respectivamente.

CONCLUSIONES

1. El 30,0% de los pacientes con candidemia presentan una forma “complicada”, definida como un episodio que causa metástasis sépticas o que es responsable de la muerte del paciente. Ningún elemento clínico es capaz de diferenciar entre pacientes con candidemia complicada y no complicada.
2. En comparación con los hemocultivos de control o el 1→3 β-D-glucano, la realización de T2MR de control en pacientes con candidemia podría predecir mejor a los pacientes con candidemia complicada, incluso aquellos con hemocultivos de control negativos.

3. Entre toda la población de pacientes que recibieron un tratamiento antifúngico empírico, tan solo el 28% de ellos tuvo una mala evolución clínica (candidiasis invasiva probada o muerte en los primeros 7 días).
4. En pacientes con sospecha de candidiasis invasiva en los que se ha iniciado un tratamiento antifúngico empírico, la determinación precoz del T2MR en combinación con los cultivos tradicionales puede, con una alta capacidad discriminatoria, identificar a los pacientes con mala evolución clínica.
5. Esta estrategia puede permitir de identificar a los pacientes que realmente pueden beneficiarse de un tratamiento antifúngico más largo.
6. En un hospital con gran experiencia en políticas de antifúngicos, la implementación sistemática de un “check-list” que incluya recomendaciones estructuradas, dirigidas a garantizar la adherencia a las guías clínicas, es capaz de mejorar el pronóstico de los pacientes con candidemia disminuyendo de forma significativa la mortalidad a 14 y 30 días.
7. En un grupo no seleccionado de pacientes hospitalizados que reciben triazoles o equinocandinas, existe una correlación débil entre la dosificación antifúngica estándar, basada en las guías, y las concentraciones séricas adecuadas del fármaco, con una gran proporción de pacientes fuera del rango terapéutico (~ 30%).
8. Una concentración sérica adecuada del antifúngico (y non una dosis adecuada según las guías) parece predecir mejor el desenlace clínico de

los pacientes, lo que aconseja la realización de TDM de antifúngicos de forma rutinaria.

9. En el estudio multicentrico retrospectivo que incluya una población de pacientes con hepatopatía grave, la frecuencia de daño hepático agudo debido a antifungicos fue del 10%.
10. La incidencia de daño hepático agudo fue igual entre los pacientes que recibieron azoles (10%), micafungina (10%) u otras candinas (10%)
11. Durante el período de seguimiento, sólo 1 paciente, que previamente había recibido tratamiento con azoles, desarrolló un hepatocarcinoma.
12. La tasa de aspergilosis invasora de brecha en una población de pacientes hematológicos que reciben profilaxis con micafungina es tan solo del 2,7%.
13. En este grupo de paciente, la determinación del galactomannano sérico como prueba de depistaje de aspergilosis invasora es un enfoque diagnóstico inadecuado debido a su bajo valor predictivo positivo y la alta tasa de resultados falsos-positivos. Sin embargo, la prueba del galactomannano sérico sigue siendo útil como test diagnóstico de aspergilosis invasora en pacientes con síntomas clínicos.

Palabras claves: Candidiasis invasora, candidemia, Aspergilosis invasoras, antifungicos, antifungal stewardship, Infecciones nosocomiales, T2 Candida Resonancia Magnetica, 1-3 β-D-glucano, *Aspergillus, Candida*

ABSTRACT

INTRODUCTION

In recent years, invasive fungal infections (IFI) are regarded as a significant health problem, mainly due to their high morbidity, mortality and high resources utilization, both for prevention and monitoring. In addition, these infections represent a continuous challenge for clinicians since there are many unmeet needs that include diagnostic, therapeutic and preventive aspects.

OBJECTIVES:

- **First objective**: To assess the usefulness of follow-up T2MR in comparison with blood cultures and the (1-->3)-beta-D-glucan assay for the early differentiation of patients with complicated or uncomplicated *Candida* BSI
- **Second objective**: To assess the potential role of T2Candida MR (T2MR) and serological biomarkers [*Candida albicans* germ tube antibodies (CAGTA) or (1-->3)-beta-D-glucano (BDG)], alone or in combination with standard cultures, for identifying patients with suspected invasive candidiasis, who may benefit from maintaning antifungal therapy.
- **Third objective:** To assess the clinical impact of a comprehensive care bundle for the management of candidemia.
- **Fourth objective:** To determine whether doses of antifungal drugs accurately predict adequate serum concentration in a non-selected group of

hospitalized patients. To evaluate the impact of inadequate dosage or inadequate antifungal serum concentrations on clinical outcome.

- **Fifth objective:** To determine the association between exposure to micafungin, other echinocandins, or azoles and the development of short-term or long term liver injury in a population of patients with pre-existing Child-Pugh B or C ESLD.
- **Sixth objective:** To assess the diagnostic performance of sGM in hematology patients receiving prophylaxis during high-risk episodes of invasive aspergillosis with micafungin and to describe the rate of breakthrough invasive aspergillosis in this population.

RESULTS:

First objective: From January to May 2017, 30 candidemic patients were enrolled in the study. Of those, 9 (30%) had complicated candidemia. Values of persistently positive samples to predict complicated episodes were respectively for BCs, T2MR and BDG as follows: Sensitivity (44.4%, 100%, 100%); Specificity (76.1%, 76.1%, 38.9%); PPV (44.4%, 64.2%, 40.9%) and NPV (76.1%; 100%, 100%). At multivariate analysis, having a positive T2 MR within the first 5 days was associated with an almost 37-fold higher risk for developing complicated candidemia.

Second objective: Overall, 14 out of 49 patients with a suspicion of invasive candidiasis (29%) had a poor outcome (7 died within the first 7-days of antifungal therapy, whereas 7 ended with a diagnosis of invasive candidiasi). CAGTA [3/14

(21.4%) versus 8/35 (22.9%), p= 1] and BDG [8/14 (57.1%) versus 17/35 (62.9%), p=0.75] results were similar in poor and good-outcome patients. Conversely, a positive T2MR was associated with a higher risk of poor outcome [5/14 (35.7%) versus 0/35 (0.0%) p=0.0001]. Specificity and PPV of a positive T2MR for predicting poor outcome were both 100%, with a NPV of 79.6%. After combining biomarkers and T2MR and with standard cultures, the combination of T2MR/standard culture showed a high capacity to discriminate patients with poor outcome from those with good clinical evolution.

Third objective: Overall, 109 patients with candidemia were included, 56 in the pre-intervention and 53 in the post-intervention period. Overall, compliance with *Candida* bundle significantly improved between pre (27/56, [48.2%]) and post-intervention (43/53, [81.1%]; p=0.01) period. Multivariate analysis revealed that being managed according to candidemia bundle had a favorable impact on 14-day mortality (HR 0.08, 95%CI 0.01-0.45, p=0.02) and 30-day mortality (HR 0.40, 95%CI 0.18-0.89, p=0.02).

Fourth objective: During the study period, 84 patients (65.4% male, 59.6 years) received antifungals for prophylaxis (40.4%), targeted (31.0%) and empirical therapy (28.6%). The most frequent drug was micafungin (28/84; 33.3%), followed by fluconazole (23/84; 27.4%), voriconazole (15/84; 17.9%), anidulafungin (8/84; 9.5%), posaconazole (7/84; 8.3%) and caspofungin (3/84; 3.6%). Considerable interindividual variability was observed for all antifungals with a large proportion of the patients (64.3%) not attaining adequate through serum concentrations, despite receiving an adequate antifungal dose. Attaining on-target serum antifungal level

was significantly associated with a favorable clinical outcome (OR=0.02; 95% CI 0.01–0.64; P = 0.03), whereas the administration of an adequate antifungal dosage was not.

Fifth objective: Twenty of 2,335 (0.85%) patients with chronic liver disease were found to have Child-Pugh B or C liver disease and received micafungin. STLI due to AF developed in 10% in each group. Most STLI were asymptomatic, and AFs had to be switched to another class in only 2 patients (1 micafungin and 1 azole). No patients developed acute liver insufficiency or had to undergo transplantation. During the follow-up period, LTLI was observed in 1 patient, who had previously treated with azoles.

Sixth objective: Among 146 evaluable high risk episodes reciving micafungin prophylaxis, 4 GM determinations were considered as true-positive in the context of probable breakthrough Invasive aspergillosis (IA) (incidence of breakthrough IA, 2.7%); 111 high-risk episodes (76%) were considered true-negative and 31 (21.2%) false-positive. No false-negative episodes were detected. All but 1 of the false-positive episodes were detected in surveillance GM tests, leading to high-resolution CT scans in 25%, all of which were negative. The positive predictive and negative predictive values of sGM for surveillance and diagnostic approaches were 3.1% and 100% and 75% and 100%, respectively.

CONCLUSIONS:

1. Thirty percent of candidemic patients fulfill the criteria for complicated candidemia, defined as an episode that 1) caused septic metastasis and/or 2) was the cause of the patient's death.
2. T2Candida test performed in patients with proven candidemia may be a better marker of complicated infection than follow-up BC's or BDG.
3. Overall, among all patients who receive empirical antifungals for clinical suspicion of invasive candidiasis, only 28% of them have a poor clinical outcome
4. In our cohort of patients with a presumed diagnosis of IC, T2MR, in combination with standard cultures, demonstrates a high discriminative ability for identifying patients with high risk of dying or developing IC.
5. Taken in the context of other clinical and microbiological tests, this new diagnostic tool may be of significant utility to identify patients who may really benefit from antifungal therapy
6. The introduction of a comprehensive candidemia bundle with antifungal stewardship program involvement significantly improved adherence to quality indicators, overall management and clinical evolution of patients with candidemia.
7. In a non-selected group of hospitalized patients receiving antifungals, there is a poor correlation between standard antifungal dosage and adequate serum concentration with a considerable proportion of patients having a low drug concentration.

8. Attaining on-target serum antifungal level significantly correlate with a favorable clinical outcome. Conversely, the administration of an adequate antifungal dosage according to current guidelines is not associated with a favourable clinical outcome.
9. En el estudio multicentrico retrospectivo que incluya una población de pacientes con hepatopatía grave, la frecuencia de daño hepático agudo debido a antifungicos fue del 10%.
10. Overall, 10% of patients with end stage liver disease receiving antifungals develop a short liver injury with no differences regarding the antifungal drug administered.
11. During the follow-up period, the administration of micafungin therapy to patients with end stage liver disease did not imply a higher risk of developing long term liver injury.
12. The incidence of breakthrough invasive aspergillosis in high risk hematological patients receiving micafungin prophylaxis is of 2.7%
13. Surveillance of asymptomatic patients receiving prophylaxis with micafungin using sGM is unnecessary, because the results are either negative or false-positive. However, sGM remains useful in the diagnosis of breakthrough IA in symptomatic patients during prophylaxis.

Keywords: Invasive candidiasis, candidemia, invasive aspergillosis, antifungals, antifungal stewardship, nosocomial infection, T2 Candida Magnetic resonance, 1-3 β-D-glucan, *Aspergillus, Candida*